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Impacts of selected leguminous tree species and kaolinite pre-amendment on oil-contaminated soil for bioremediation in the oil-bearing region of Nigeria

A thesis submitted for the degree of

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By

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A Thesis Submitted in partial fulfilment of the requirements of the University of Wolverhampton for the degree of Doctor of Philosophy.

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ABSTRACT

This study investigates the impacts of selected Leguminous Tree Species (LTS) and kaolinite pre-amendment on oil-contaminated soil. It covered assessment of different levels of contamination (0, 25, 50, 75 and 100 ml in 4000 g soil; which represents the degree of light crude oil spillage concentration as 0.0, 0.63, 1.25, 1.88 and 2.5 %v/w) on the growth performance of *Albizia adianthifolia*, *Albizia odoratissima*, *Bauhinia monandra*, *Delonix regia*, *Peltophorum pterocarpum* and *Tetrapleura tetraptera* LTS investigated. Percentage germination, seedling height, seedling girth, number of leaves and number of nodules decreased as the concentrations of crude oil in soil samples increased. LTS affected soil physicochemical properties. Soil acidity decreased; soil organic matter, carbon content and exchangeable ions increased. N, P and K were altered in the LTS planted soil as compared to controls, but there were no significant ($P > 0.05$) differences. There were increased microbial counts in the crude oil-contaminated soil planted with LTS as compared with non-LTS planted soils. Hydrocarbon removal was significantly higher ($P < 0.05$, $n = 3$) in LTS planted soil than in non-planted soil. *D. regia* planted soils had most hydrocarbon removal and had significantly more growth in terms of plant height, girth and leaf production in the field. Kaolinite (10 and 20 g samples) applications were suitable and effective sorbent agents for oil-contamination at the different oil concentrations. The sorption potential of kaolinite increased with the increase in kaolinite to 20 g. The potential re-usability of kaolinite after the initial use for oil sorption was analysed and 10 g of burnt kaolinite sorbed 43.62%, while 20 g sorbed 58.90%. The rate of oil sorption by fresh kaolinite was significantly higher than burnt kaolinite. Results show the considerable potential of phytoremediation protocols with LTS and kaolinite as combined remediating agents for oil spill remediation in the humid tropics.

DEDICATION

This research work is dedicated to:

- My loving parents, **Elder Joseph Olalere** and **Chief Beatrice Adenike Oyedeji**, who financed my early educational trainings with their hard earned teaching incomes and thus, gave me a solid educational foundation.
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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
ANOVA	Analysis of Variance
AO	Automobile Oil
BD	Bulk Density
BPW	Buffered Peptone Water
CFU	Colony Forming Unit
CoV	Co-efficient of Velocity
DF	Dilution Factor
DPR	Department of Petroleum Resources
EC	Electrical Conductivity
FAO	Food and Agriculture Organization of the United Nations
FEMA	Federal Environmental Protection Agency
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrophotometry
Gt	Germination
HBC	Heterotrophic Bacteria Count
HFC	Heterotrophic Fungi Count
IR	Infrared Radiation
ITRC	Interstate Technology and Regulatory Council
IZA	International Zeolite Association
LoC	Level of Contamination
LSD	Least Significant Difference

LTS	Leguminous Tree Species
MC	Moisture Content
NA	Nutrient Agar
NNPC	Nigerian National Petroleum Corporation
PAH	Polyaromatic Hydrocarbon
PC	Personal Computer
PDA	Potato Dextrose Agar
PS	Particle Density
SD	Standard Deviation
SEM	Scanning Electron Microscopy
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SP	Soil Porosity
SPSS	Statistical Package for the Social Sciences
SQLs	Soil Quality Indicators
SUB	Secondary Building Unit
TOC	Total Organic Carbon
TOM	Total Organic Matter
TPH	Total Petroleum Hydrocarbon
UoW	University of Wolverhampton
USD	United States Dollar
USEPA	United States Environmental Protection Agency
WAP	Weeks After Planting
XRD	X-Ray Diffraction Spectrometry
XRF	X-Ray Fluorescence Spectrometry.

CHAPTER ONE

Introduction and Literature review

1.1. Background to the study

Crude oil is a mixture of hydrocarbon compounds and a vital non-renewable natural resource that has emerged as a prominent commodity in the local and international market with considerable socio-economic benefits for producing nations. Nigeria is one of the foremost crude oil producing countries in the world. Crude oil has been an important part of the Nigerian economy for decades, since large reserves were discovered in the 1950s, but the monetary returns the country has derived from it has environmental consequences (Anoliefo *et al.*, 2006a; Brandt *et al*., 2006). It has taken central stage as the foremost source of energy, as well as serving as the basis for the production of energy and primary raw material for petro-chemicals and allied industries, and is the main source of fuel (White *et. al*., 2006). The great demand and utilization of petroleum products have encouraged its exploration and exploitation in Nigeria and globally and this increase in world production, refining and distribution of petroleum products has brought with it many problems of environmental contamination, which have impacts on ecosystems. Crude oil pollution resulting from spillage is one of the foremost environmental problems associated with petroleum exploration, production and marketing in Nigeria. The increase in demand for petroleum products have generated a corresponding increase in pollution incidents and some of the effects include damage to terrestrial and aquatic life, changes in water quality and destruction of the aesthetic value of beaches, loss of soil fertility and agricultural productivity and considerable environmental and health hazards (Escalante-Espinosa *et al*., 2005; Denys *et al*., 2006; Zand *et al*., 2010; Bamidele and Igiri, 2011; Oyedeji *et al*., 2012).

Soil is an essential natural resource which acts as a focal point for ecosystem dynamics. It is a multicomponent and multifunctional system, which has identifiable operating limits and a characteristic spatial configuration. Soil system is capable of supports agricultural production and other ecosystem services (Kibblewhite *et al*., 2008). The various components of soil and the environment interact to continuously provide several essential ecosystem services (such as food, fuel and fibre), which are required to support organisms living in the environment, water regulation, carbon and nutrient cycling, soil structure maintenance and regulation of pests and diseases (Kibblewhite *et al*., 2008; Kassam, 2009). Due to the utilization of soil for more needs, goods and services, largely owing to urbanization, industrialization, exploration of natural resources (such as crude oil) and population growth, there is growing land degradation. This involves landslides, soil erosion, desertification, organic matter decline and contamination (European Commission, 2002). Contaminated soils and sites have some level of contaminants present in them and damages ecosystems (Oyedeji *et al*., 2012). Soil contamination has been recognized as one of the major threats to the environment. The substances that can contaminate soils include the spillage of crude oil, petroleum products, heavy metals, mineral pollutants, dumping of organic wastes, pesticides and other pollutants (Nwaugo 2006; Osam *et al*., 2011a). Due to the fact that pollutants exist in various forms, there is no common solution to solve all kinds of soil contamination.

Crude oil spillages into aquatic and terrestrial ecosystems have been extensive, significant (Ogboghodo *et al*., 2004) and often occur during crude oil drilling, burst pipelines due to high pressure, corrosion or sabotage of pipes, tank overflows, tank loading operation failure, leakages and seepages (Osuji and Onojake, 2004; 2006;

White *et al*., 2006; Tanee and Kinako, 2008; Aroh *et al*., 2010). These can cause poisoning of drinking water and destruction of aquatic and terrestrial vegetation. Crude oil contamination of agricultural soils generally decreased plant growth and productivity (Anoliefo *et al*., 2006a; Kayode *et al*., 2009a); although, growth in some plants is possible with low levels of oil contamination in the soil (Amakiri and Onofeghara, 1984; Huang *et al*., 2004). However, the reasons for reduced plant growth are numerous and these reasons range from direct toxic effects of oil on plants (Amadi *et al*., 1996), lack of germination due to non-viable seeds (Rowell, 1977) or reduced germination arising from toxic soil conditions (Anolifo *et al*., 2001). Oil can enter the seed and disturb metabolic reactions or kill the embryo (Adam and Duncan, 2002), low water uptake and reduced nutrient availability (Merkl *et al*., 2005a), osmotic stress and root gas exchange (Ko and Day, 2004; Merkl *et al*., 2005b; Robertson *et al*., 2007) to poor soil aeration and porosity (Kayode *et al*., 2009b).

There are adverse effects of oil contamination on annual food crops (Isirimah *et al*., 1989; Kayode and Oyedeji, 2012). At high concentrations of oil in soil, most plant species suffer serious decreased growth (Amakiri and Onofeghara, 1983; Anoliefo and Okoloko, 2000; Kayode *et al*., 2009a). The effect of oil spillage on agricultural soil is of great concern and requires solutions. Oil contamination affects considerable proportions of agricultural soils in oil-producing nations. Oil spillage gives indigenous dwellers and farmers considerable concern because their crops are often considerably affected. The oil companies also are not left out of the oil spillage menace, as they pay huge financial resources as reparation to farmers for damaged crops and farmlands.

Oil pollution has been a major environmental concern in many countries of the world for many years. For instance, Lakeview Gusher oil spillage occurred in California and spilled ~1, 200,000 tonnes of crude oil between May 1910 and September 1911 and was acclaimed the largest crude oil spillage ever or at that time (Wang *et al*., 2011). It is estimated that in Saskatchewan alone there are several hundred sites contaminated with petroleum hydrocarbons (Frick, *et al*, 1999). Other notable oil spill occurrences in the world are summarized in **Table 1.1.**

Table 1.1: Major oil spills in the world by order of quantity in barrels (adapted from Eja and Ogri, 2003; Wang *et al*., 2011)

Date	Location	Spill type	Tonnes of crude oil	Barrels
14/5/1910-10/0/1911	U.S. Kern County, California	Lakeview Gusher	1,200,000	9,000,000
20/4/10-15/7/10	U.S. Gulf of Mexico	Deepwater horizon	560,000-585,000	4,100,000-4,300,000
23/1/1991	Iraq, Persian Gulf and Kuwait	Gulf water oil spill	270,000-820,000	2,000,000-6, 000,000
3/6/1979-23/3/1980	Mexico, Gulf of Mexico	Ixoc I	454,000-480,000	3,329,000-3,520,000
19/7/1979	Trinidad and Tobago	Atlantic Empress/ Aegean Captain	287,000	2,105,000
2/3/1992	Uzbekistan	Fergana Valley	285,000	2,090,000
4/2/1983	Iran, Persian Gulf	Nowruz Field Platform	260,000	1,907,000
28/5/1991	Angola	ABT Summer offshore	260,000	1,907,000
6/8/1983	South Africa, Saldanha Bay	Castillo de Bellver	252,000	1,848,000
16/3/1978	France, Brittany	Amoco Cadiz	223,000	1,635,000
11/4/1991	Italy, Mediterranean sea near Genoa	MT Haven	144,000	1,056,000
10/11/1988	Canada	Odyssey	132,000	968,000
24/3/1989	U.S. Prince William Sound, Alaska	Exxon Valdez oil tanker	35,065-–103,896	257,000-750,000

Similar spills and environmental contaminations have followed oil exploration in Nigeria. Nwankwo and Ifeadi (1983) reported a pipe burst in Exxon Mobil, Idoho platform in Rivers State, which resulted in the discharge of ~40,000 barrels of oil into the Atlantic coastline of Nigeria. The most serious of the oil spills in the Niger Delta region of Nigeria occurred in July 1979, when a storage facility at the Shell operated Forcados Terminal collapsed, spilling an estimated 580,000 barrels of crude oil into the surrounding land, mangrove swamps and waters (Nwankwo and Ifeadi, 1983). This problem has now taken serious dimensions in terrestrial and aquatic environments in many developing countries, such as Nigeria. Whenever there is an occurrence of on-shore oil spills, the soil ecosystem is usually contaminated by crude oil, leading to serious fires that may consume arable land (Osuji, 2001; Oyedeji *et al.*, 2012). Many terrestrial ecosystems and shorelines in the oil-producing communities cover important agricultural lands and are under continuous cultivation and farming activities (Osam *et al*., 2008).

In the present era of proliferated environmental awareness and government regulation, efforts are geared towards cleaning-up contaminated sites and soils with better approaches and management. Environmental managers can choose from several approaches to remediate petroleum-contaminated soil and ground-water. These approaches range from intensive engineering techniques to natural attenuation. A few approaches rely entirely on natural processes to remediate sites with no human intervention, while others depend largely on biological processes, such as bioremediation.

As part of the exigency programmes, oil producing and prospecting companies had been effecting clean-up (remediation) measures in some cases in oil-spilled soils, but petroleum hydrocarbons still persist in the soil environment long after the purported remediation, thus impairing agricultural productivity (Osam *et al*., 2008). The remediation method(s) adopted in such clean-ups has hitherto been traditional practices, such as the 'pump-and-treat' and 'dig-and-dump' techniques, which are often expensive, have limited potential, make the soil infertile (since they destroy the environment) and are usually only applicable to small areas (Rivera-Cruz, 2004).

To curb environmental contamination resulting from oil spillage in an environmentally-friendly manner and to ensure cost-effectiveness, good bioremediation processes are now practised in many countries. Bioremediation is the use of organisms (plants, animals or micro-organisms) to transform harmful substances to a non-toxic states. It has become an alternative to physical and chemical methods of remediation, due to its numerous advantages. Bioremediation can be less expensive, achieve complete degradation of recalcitrant organics and can be used *in situ* for pollutants, even at low concentrations (Anoliefo *et al*., 2006b). Consequently, in this era of global economic recession, there is an imperative need for a further improved, but natural clean-up methods, known as 'phytoremediation' (Schröder *et al*., 2002; Brandt *et al*., 2006). This involves green plants and associated micro-biota, soil amendments and agronomic techniques to decrease the effects of environmental contaminants.

Phytoremediation is a biological remediation (bioremediation) strategy that involves the use of living plants, often with soil amendments with associated microbes in the root system of plants for the removal, degradation, extraction and detoxification of contaminants (both organic and inorganic) in soils, sludge, sediments, air and ground-water (White *et al.,* 2006) by absorbing, translocating or sequestering organic contaminants and removing them from the soil compartment (Cunningham *et al*., 1996). This method is appropriate for heavy metals, radionuclides and a wide range of organic contaminants such as petroleum hydrocarbons (Schroder *et al*., 2002). Phytoremediation process is effective at temperatures of (~37º-40ºC) and with adequate water supply and nutrients. Hence the method can be very effective in tropical countries (Brandt *et al*., 2006). The use of plants in soil remediation and unconfined ground-water is appealing for several reason:

1. Plants provide a remediation strategy that utilizes solar energy.
2. Vegetation is aesthetically pleasing.
3. Plant can be harvested and tested as indicators of the level of remediation.
4. Plants help contain the region of contamination by removing water from soil.
5. Rhizosphere microbial communities biodegrade a wide variety of organic contaminants.
6. Many plants have mechanisms for transporting oxygen to the rhizosphere (Shimp *et al.,* 1993).

Before effective plant remediation strategies can be developed, an understanding is required of the physical, chemical and biological relationships that determine the fate of each contaminant in the rhizosphere. Phytoremediation is a non-destructive, cost-effective *in situ* technology that uses plants and their associated micro-organisms, to

clean up contaminated soils and is, therefore, appropriate and useful in environmental and ecological research (Nie *et al.,* 2011). It has become a practical solution for the remediation of petroleum hydrocarbon polluted sites (Frick *et al.*, 1999; Tanee and Kinako, 2008). Plants that can grow well in crude oil contaminated soil have been described as potentially suitable species for the phytoremediation of crude oil-polluted soil (Bamidele and Agbogidi, 2006).

In recent times, soil amendments (such as zeolites, fertilizers, sawdust and manure) have been utilized in remediation processes. Merkl *et al*., (2005c); Brandt *et al*., (2006); Tanee and Kinako, (2008); Chorom *et al.,* (2010) found application of fertilizers useful in bioremediation processes of crude oil contamination. The role of natural zeolites, such as clinoptilolite in agriculture, essentially in the growth of plants (Leggo, 2000; Manolov *et al.,* 2005; Leggo, *et al.,* 2006) and soil system improvement through its use as soil-amendment (Manolov *et al*.,2005; Adbi, *et al*., 2006; Földesová *et al.,* 2007) and for improved soil physico-chemical properties have been identified. The natural zeolites, such as clinoptilolite, are also effective absorbents of petroleum products (Misaelides, 2011). Kaolin is zeolite precussor and the primary clay mineral material present in the kaolinite mineral group (Brigatti *et al.*, 2006). The important uses of kaolinite are in the industrial sector, such as the paper industry, paint production, production of ceramics, sanitary ware and electrical porcelain (Edomwonyi-Out *et al*., 2012). It can also be used in synthetic zeolites production (Xu *et al*., 2007). In the current research, the impact of leguminous tree species on crude oil contaminated soil and natural zeolites precussor, particularly kaolinite, as suitable pre-treatment and soil amendment for oil spillage remediation is investigated.

The technology behind phytoremediation is ecologically friendly, solar-energy driven, and is based on the concept of using "nature to cleanse nature". It is the intention of this study to evaluate the efficacy of selected Leguminous Tree Species (LTS) commonly found growing in the oil-bearing regions of Nigeria, with a view to determining their tolerance and effectiveness in phytoremediating crude oil contaminated soils. The study will also investigate the potential role of kaolinite in enhancing the remediation of oil spillages.

1.2. Statement of the problem

The exploration, exploitation, refining, and transportation of petroleum resources in the onshore and offshore have the potential to cause diverse environmental problems, such as crude oil and petroleum-by products spillage (Osuji, 2001; Anoliefo *et al*., 2006a). Thus, ecosystems are polluted as a result of the onshore and offshore activities of oil companies.

The so-called clean up measures adopted by these operating companies are less effective as significant amounts of petroleum hydrocarbons have been found in such supposedly cleaned up sites (Osam, *et al*, 2008). The ineffectiveness of the remediation methods have been revealed by the retarded growth of plants in such purported remediated oil-spilled soils, as well as chlorosis of leaves, dehydration of plants, and the attendant low agricultural yield that still manifests many years after the spill and clean-up. Also, contaminants claimed to have been so removed are not wholly flushed out of the soil ecosystem; they are merely removed or transferred from one part of the soil environment and deposited into another. In some cases,

chemicals are added in an attempt to remove contaminants, hence spreading the pollutants or adding more pollutants (USEPA, 2001).

These old methods of remediation, dubbed ‘pump-and-treat’ and ‘dig-and-dump’ techniques are often expensive, have limited potential, and are usually only applicable to small areas. Additionally, the techniques often make the soil infertile and unsuitable for agriculture and other uses by destroying the environment. Hence, there is the need to develop and apply alternative environmentally-friendly innovations, such as phytoremediation strategies to accelerate the contaminated soil recuperation process after pollution incidents. The rationale for this work is based on a series of remediation efforts in Nigeria which do not offer the desired results for the recovery and sustainability of ecosystems (Tanee and Kinako, 2008). However, it is hoped that phyto-remediation of crude oil-contaminated soil with kaolinite pre-amendment will offer a remediation solution for such polluted terrestrial habitats. Therefore, the study investigates environmental contamination, with crude oil, tolerance and/or degradation level of LTS in crude oil-contaminated soil, potentials of LTS as possible phytoremediation agents, evaluation of sorption potentials of kaolinite resources as suitable soil-amendments and the development of ecosystem remediation technology for crude oil-contaminated soil. The commercialization of this innovation will be of economic value to Nigeria and this study will contribute directly to terrestrial ecosystem management.

The aims of this study are:

1. To determine the effects of crude oil contamination on LTS germination and yield performance (height, girth, leaf production and nodulation) and selected physical, chemical and biological soil properties.
2. To assess the tolerance and/or degradation level of LTS in crude oil contaminated soil.
3. To evaluate the efficacy of leguminous tree species in enhancing remediation of crude oil contaminated terrestrial soils, with and/or without soil amendments.
4. To evaluate the use of natural zeolites (such as kaolinite) as potential soil amendments for oil sorption and remediation processes.
5. To develop an ecosystem remediation technology for crude oil contaminated soils which may be of economic value to Nigeria, and beyond.

1.3. Literature review

This section reviews literature related to the study under the following headings:

- Composition of crude oil.
- Contamination of soil ecosystem with crude oil and petroleum-based-products.
- The oil-bearing region of Nigeria.
- Discovery of crude oil in the Niger Delta region and Nigerian economy
- Terrestrial oil spillages in Nigeria.
- Nigerian government legal structure on environmental pollution with crude oil.

- Remediation technologies for crude oil-contaminated soils.
- The concept of phytoremediation.
- Tolerance mechanism(s) of plants and their suitability for remediation.
- Selection of plant species for phytoremediation.
- Suitability of *Fabaceae* for phytoremediation of hydrocarbon contaminated soils.
- Comparison of phytoremediation with conventional strategies.
- General overview of soil-amendments in the remediation of contaminated soils.
- An overview of kaolinite and its deposition in Nigeria.
- An overview the selected LTS.

1.4. Composition of crude oil

The composition of each oil reserve is unique, varying in different oil-producing regions and even in different unconnected zones of the same formation (NRC, 1985). Oil composition also varies with the amount of refining and the products of its fractional distillation (**Table 1.2**). Significantly, many oil compounds differ markedly in volatility, solubility and susceptibility to biodegradation (Atlas, 1988). Petroleum hydrocarbons can be grouped into four broad categories; saturates, aromatics, asphaltenes and resins (Leahy and Colwell, 1990).

Table 1.2: Products of fractional distillation of petroleum Atlas, (1988) described fractional distillation of petroleum as follow:

Fractions	Composition	Boiling point range (ºC)	Uses
Natural gas Naphtha	C_1–C_4	<20	Fuels
Petroleum ethers	C_5–C_7	20–60	Solvents
Petrol	C_6–C_{12}	50–230	Fuels
Kerosene	C_{11}–C_{16}	200–300	Fuels
Gas oil	C_{13}–C_{16}	280–360	Fuels
Lubricating oil	> C_{20}	350–430	Lubricants
Fuel oil residue	Hydrocarbon of high molecular weight	>400	Asphalt for roads, grease

(Source: Atlas, 1988).

1.5. Contamination of soil ecosystems with crude oil and petroleum-based-products

Major points of soil pollution with refinery products are petrol stations, service stations and seaports (Wyszkowski, *et al*, 2004). The release of petroleum and refinery products into the soil environment causes soil degradation. Petroleum-based products initiate a series of processes, affecting both biotic and abiotic elements in an ecosystem (Bamidele and Igiri, 2011). Crude oil and its derivatives are composed of aliphatic, oleic, naphthenic and aromatic hydrocarbons, which modify physical and chemical properties of soil and its structure (Atlas, 1988). These compounds are largely responsible for changes in soil fertility. Soil polluted by petroleum-based products losses its biological activity and may take a considerable time to recover without remedial measures (Tanee and Kinako, 2008).

1.6. The oil-bearing regions of Nigeria

The Niger Delta region is the main oil-bearing region of Nigeria. It is one of the most resourceful deltas in the world and approximately corresponds to the south-south geopolitical zone (Anifowose 2008; Omofonmwan and Odia, 2009). The Niger Delta region covers the states of Akwa-Ibom, Bayelsa, Cross Rivers, Edo, Delta, Imo, Ondo and Rivers State (**Figure 1.1**). Recently, crude oil has been discovered in Anambra and Lagos States. The Niger Delta is located in the Atlantic coastal area of southern Nigeria and is the second largest delta in the world, with a coastline of ~450 km which ends at the entrance of the Imo River (Kadafa, 2012). The region occupies ~20,000 km^{-2} and it is the largest wetland in Africa and one of the three largest wetlands in the world (Ohimain, 2003; Anifowose, 2008; Eregha and Irughe, 2009).

The Niger Delta area consists of rivers, creeks and estuaries estimated at 2,370 km^{-2} and stagnant swamps cover ~8,600 km^{-2}.

The region is a typical tropical rainforest with ecosystems richness in diverse species of flora and fauna both aquatic and terrestrial (Ohimain, 2003). Research reports and the result of a census conducted by the National Population Commission (1991) estimated that ~25 % (30 million) of the total Nigerian population lives within the Niger Delta region (Twumasi and Merem, 2006; Uyigue and Ogbeibu, 2007). Due to the agricultural, industrial and economic richness and uniqueness of the Niger Delta region, the area attracts much attention. Ecosystems management in the region is of great concern to the people of the area and the Nigerian government.

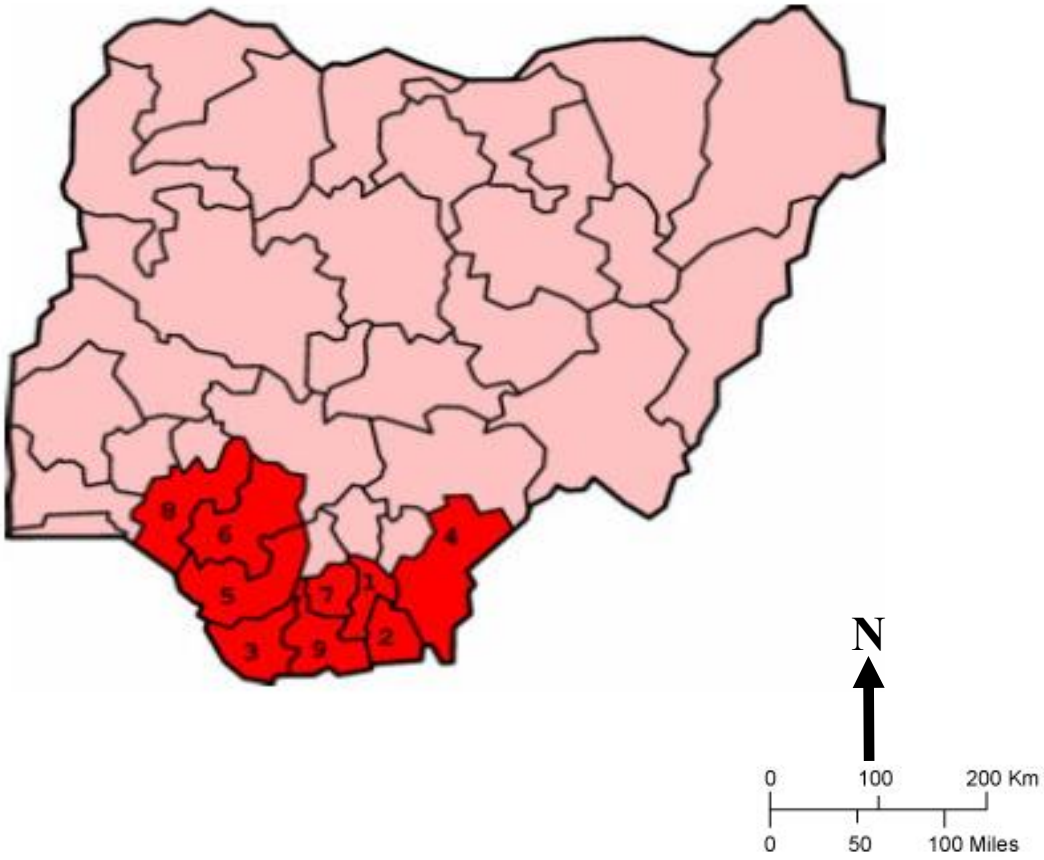


Figure 1.1: Map of Nigeria showing the Niger Delta Region and numerically indicating the location of its member states Abia[1], Akwa Ibom[2], Bayelsa[3], Cross River[4], Delta[5], Edo[6], Imo[7], Ondo[8], Rivers[9] (Source: Erakhrumen, 2007).

1.7. Discovery of crude oil in the Niger Delta region and Nigerian economy

The British discovered crude oil in the Niger Delta region in the late 1950s and it was discovered at a commercial scale by Shell British Petroleum, which is now called Royal Dutch Shell. The discovery was at Oloibiri, a village in the present day Bayelsa State and its commercial production began in 1958 (Okoh, 2003) with the production of ~6,000 barrels a day (Nwilo and Badejo 2006; Uyigue and Ogbeibu, 2007). The region has large oil and gas reserves, and has been ranked as the sixth world's largest exporter of crude oil (Omofonmwa and Odia, 2009). About 90% of the major oil export of Nigeria comes from the region and accounted for ~80% of government revenue since 1981. The overall contribution of the oil sector to the national economy grew from 84% in 2000, to 95% in 2002 to ~96.7% in 2003 (Twumasi and Merem, 2009). The Niger Delta has emerged as one of the most ecologically sensitive regions in Nigeria. Since the discovery of oil in the region, the economy of the country has been boosted greatly. The Niger Delta is highly susceptible to adverse environmental changes, arising from the exploitation and exploration activities in the area and due to these oil activities, the area has become ecologically degraded (Odokuma and Inor, 2002; Bayode *et al*., 2011). Several militia agitations due to environmental degradation have been witnessed in the Niger Delta over recent years (Inoni *et al*., 2006). The main oil companies operating in the nation are shown in **Table 1.3.**

Table 1.3: The major oil producing companies operating in the Niger Delta region of Nigeria

Name of Company	Shareholders	Operation	National production
Shell Petroleum Development Company	NNPC – 55% Shell – 30% Elf – 10% Agip – 5%	Shell	42.0%
Mobil Producing Nigeria	NNPC – 50% Mobil – 42%	Mobil	21.0%
Chevron Nigeria	NNPC – 60% Chevron – 40%	Chevron	19.0%
Nigerian Agip Oil	NNPC – 60% Agip – 40%	Agip	7.5%
Elf Petroleum Nigeria	NNPC – 60% Elf – 40%	Elf	2.6%
Texaco Overseas (Nigeria) Petroleum	NNPC – 60% Texaco – 20% Chevron – 20%	Texaco	1.7%
Others (Non-major oil producing companies)			6.2%
			100%

(Modified after: Kadafa *et al*., 2012).

There are 606 oil fields with 355 situated onshore; 251 situated offshore, 5,284 drilled oil wells and ~7,000 km of oil and gas pipelines in the area (Anifowose, 2008; Onuoha, 2008) (**Figure 1.2)**. In 1978, the Warri refinery was established with an initial production capacity of 100,000 barrels per day and its production rate was later increased to 125,000 barrels per day in 1986 of light crude oil.

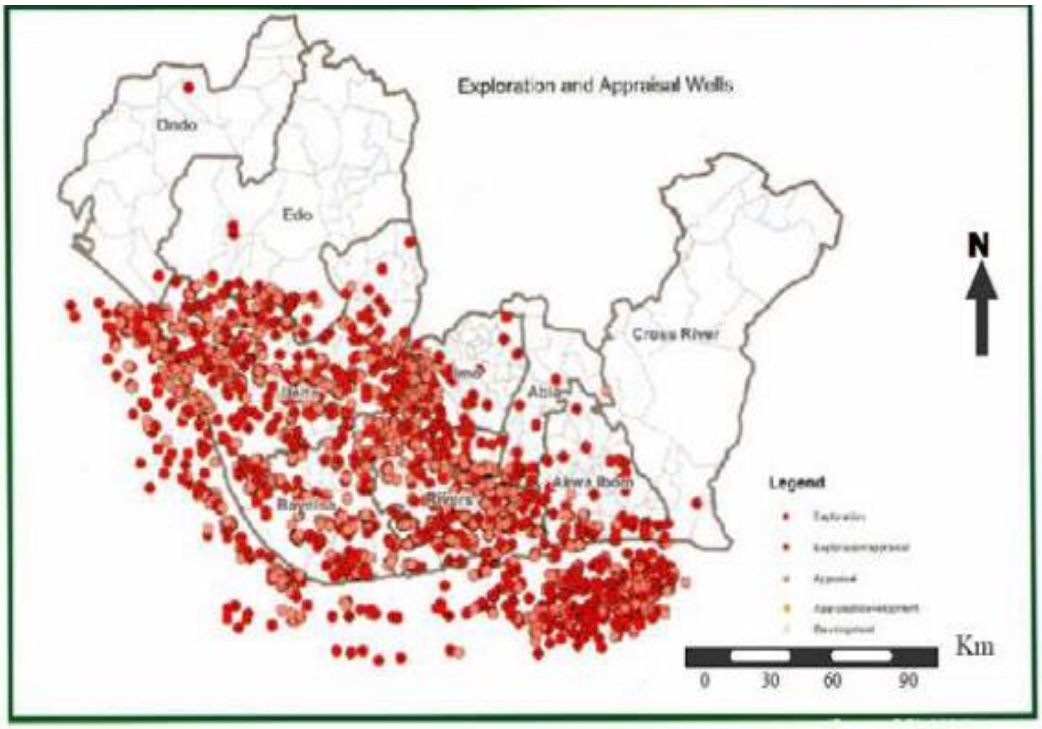


Figure 1.2: Map of the Niger Delta region showing the onshore and offshore oilfields (Source: Kadafa, 2012).

Most reserves are located along the Niger Delta River, offshore the Bright of Benin, Gulf of Guinea and the Bright of Bonny. Current exploration focuses on deep and ultra-deep offshore exploration with some activities in the Chad Basin located in north-west Nigeria. As of 2008, Nigeria's crude oil production averaged 1.94 million bbl/d making it the largest oil producer in Africa, with production slightly over 2.2 million bbl/d in 2009 (Kadafa *et al.*, 2012) and current production stands at 2.5 million bbl/d in 2015 (NNPC, 2015).

1.8. Terrestrial oil spillages in Nigeria

The oil-fed economic and industrial growth that has occurred in Nigeria has caused environmental damage (Anoliefo *et al*., 2006a). A realization that crude oil contamination of the environment is extensive and significant has emerged, largely as a result of increased awareness with improved evaluation methods. Osuno (1989) reported that Nigeria had 20 drilling rigs, over 4500 oil wells, 140 flow stations and production platforms. However, Ifeadi *et al*. (1985) reported that between 1960-1985, 1581 wells (45%) were drilled on land compared with 1196 (36%) offshore and 748 (21%) in swamps. This reveals that more oil wells were drilled on vegetated land than offshore and consequently the alarming rate of oil spillage on soil.

Crude oil spills onshore (vegetated land) are attributed to several factors, including burst pipelines due to high pressure or corrosion, tank overflows, leakages, seepage and bunk activity. Flowline/pipeline leakage accounted for >30% of the total number of occurrences of oil spills and contributed >50% of oil spilled into the environment. Pipeline and flow lines leak are most damaging (Orubiama 1983). The spills resulting from these leakages or breakages are not attended to quickly due to delay in closing downstream valves or back pressure flows. It has been estimated that between 9-13 million barrels (1.5 million tonnes) of oil has been spilled into the Niger Delta ecosystem within the last 50 years. This figure is ~50 times the quantity spilled in the Exxon Valdez oil spillage in Alaska in 1989 (CEESP-IUCN 2006) and most of the spilled oil finds its way into farmlands, dry-lands and streams. The first oil spill in Nigeria occurred at Araromi in present Ondo State in 1968 and numerous other spills have occurred since (**Table 1.4**).

Table 1.4: Oil spillage (barrel) in Nigeria and its associated effects (Sources: Environmental Right Action, 2000; Johnson, 2004; Aroh *et al*., 2010).

Year/Month	Incident	Primary cause	Quantity Spilled	Spill site	Major impacts/effects
1970	Oil blowout	Corrosion and operational failure	>250 b	Shell BP Bomu II	Air, soil and water pollution, loss of ecological and aquatic species and health problems.
1972	Oil blowout	Corrosion and operational failure	>250 b	Elf Obaji 21	Air, soil and water pollution.
1978	Oil blowout	Corrosion and operational failure	300,000 b	Gocon's Escravos	Air, soil and water pollution.
1978	Oil blowout	Corrosion and operational failure	580,000 b	SPDC Forcados Terminal	Air, soil and water pollution.
1980	Oil blowout	Corrosion and operational failure	400,000 b	Texaco Escravo Funiva	321 villages displaced, 18 people killed, environment polluted.
1982	Pipeline rupture	Sabotage by locals	18,818 b	Abudu	Air, soil and water pollution, loss of vegetation and health problems.
1986	Pipeline rupture	Sabotage by locals	18,818 b	Escravos	Villages displaced, eight creeks and villages affected, property lost.
1998	Pipeline rupture	Sabotage by locals	40,000 b	Jesse	~1,000 lives lost, air, soil and water pollution, health problems and villages displaced.
1998	Oil blowout	Corrosion and operational failure	-	Idaho	Air and water pollution, loss of flora and fauna.
1999	Pipeline rupture	Sabotage by locals	-	Ishiagu	Water and soil pollution, seven people killed, loss of arable land and health problems.
May 2000	Pipeline rupture	Sabotage by locals	-	Diebu	Water and soil pollution, loss of aquatic and ecological species, fishing activity restricted
11 July 2000	Pipeline rupture/fire	Sabotage by locals	-	Adeje	Air, soil and water pollution, loss of lives and property, health problems.
17 July 2000	Pipeline rupture/fire	Sabotage by locals	-	Jesse	Air, soil and water pollution, loss of lives and property.

01 Aug. 2000	Pipeline rupture	Sabotage by locals	-	Ishiagu	Water and soil pollution, loss of lives and property, loss of arable land.
3 January 2002	NNPC Pipeline rupture	Sabotage by locals	-	Escravos	Water air and soil pollution, ecological damage.
15 Oct. 2002	NNPC Pipeline rupture	Sabotage by locals	40,000 b	Akute Ogun State	Air, soil and water pollution, 20 people died.
16 March 2003	NNPC Pipeline rupture	Sabotage by locals	40,000 b	Forcados	Loss of lives and property, air, soil and water pollution.
18 March 2003	NNPC Pipeline rupture	Sabotage by locals	40,000 b	Ishiugu	Water, soil and air pollution, loss of lives and property.
29 April 2003	NNPC Pipeline rupture	Sabotage by locals	-	Escravos	Loss of revenue, loss of lives and property.
3 May 2003	Pipeline rupture/fire	Sabotage by locals	40,000 b	Oso	Air, soil and water pollution, loss of lives and property.
22 June 2003	Pipeline rupture/fire	Sabotage by locals	40,000 b	Isiukwato	200 people burnt to death, air, soil and water pollution, loss of arable land.
26 Sept. 2003	Pipeline rupture/fire	Sabotage by locals	40,000 b	Nembe	Five people killed, loss of revenue, water and soil pollution.
05 Aug. 2005	Pipeline rupture/fire	Sabotage by locals	40,000 b	Ishiugu	Five people killed, loss of revenue, water and soil pollution.
21 Dec. 2005	Pipeline rupture/fire	Sabotage by locals	40,000 b	Diebu Creek	Water and soil pollution, loss of revenue and ecological damage.
January 2006	Pipeline rupture/fire	Sabotage by locals	40,000 b	Brass Creek	Water and soil pollution, ecological damage.
March 2006	Pipeline rupture/fire	Sabotage by locals	40,000 b	Agere Ekeremor	Water and soil pollution, ecological damage.
May 2006	Pipeline rupture/fire	Sabotage by locals	40,000 b	Snake Island Lagos	Air, water and soil pollution, economic activity affected, 200 killed, aquatic and ecological damage.

The most recent and large oil spill occurred in Oporoma area of Bayelsa State on 11-15 May 2015 spilling 3360 barrels from the Diebu Creek-Nun River pipeline and damaging vegetation and claimed many lives. The Niger Delta has a complex and extensive system of pipelines running across the region and large amounts of oil spill incidences have occurred through pipeline and storage facility failures. These failures could be caused by material defect, pipeline corrosion, ground erosion but the oil companies blame most spills on sabotage. The Department of Petroleum Resources contends that 88% of the oil spill incidences are traceable to equipment failure, other causes of oil spills in the Niger Delta are vandalism, oil blowouts from the flow stations, accidental and deliberate releases and oil tankers at sea (Nwilo and Badejo, 2006). Considering the large quantities of oil reportedly lost to vegetated land, and the effect this had on most inhabitants, many of who are subsistence farmers, one could appreciate the crisis situation oil spillage has attained in Nigeria.

1.9. Nigerian government legal structure on environmental pollution with crude oil

The ownership of all natural resources, including crude oil, is vested in the government of Nigeria through the Constitution. The Constitution requests that every national and international company formally obtains a license at the Ministry of Petroleum Resources for the purposes of oil exploration, production, drilling, storage and refining. This implies that the Ministry is in charge of all activities relating to oil exploration in Nigeria (Kadafa *et al.,* 2012). Shortly after independence and precisely in 1963, the Federal Government of Nigeria initiated the legal structure (principal and subsidiary legislation) for the control and partial alleviation of pollution resulting from petroleum industries. Some of these legal structures include:

- Mineral Oil Safety Regulation (1963).
- Oil in Navigable Waters Regulation (1968).
- Oil in Navigable Waters Act No 34 (1968).
- Petroleum Regulations (1967).
- Petroleum Decree (1969).
- Petroleum (Drilling and Production) Regulation (1969).
- Petroleum (Drilling and Production) Regulation (1973).
- Petroleum Refining Regulation (1974).
- Federal Environmental Protection Agency Act (30 December 1988).
- Mineral Oils (Safety) Regulations (1997).

Amendments:

a) Petroleum (Drilling and Production) (Amendment) Regulations (1990).

b) Petroleum (Amendment) Decree (1996).

c) Petroleum (Amendment) Decree No. 23 (1998).

d) Petroleum (Drilling and Production) (Amendment) Regulation (1996).

There are some other relevant National and International Agreements such as:

a. Endangered Species Decree Cap 108 LFN (1990).

b. Federal Environmental Protection Agency (FEPA) Act Cap 131 LFN (1990).

c. Harmful Waste Cap 165 LFN (1990).

d. International Convention on the Establishment of an International Fund for Compensation for Oil Pollution Damage (1971).

e. Convention on the Prevention of Marine Pollution Damage (1972).

f. African Convention on the Conservation of Nature and Natural Resources (1968).

g. International Convention on the Establishment of an International Fund for the Compensation for Oil Pollution Damage (1971).

h. Oil Pollution Act (OPC) (1990).

i. Environmental Guidelines and Standards for the Petroleum Industry in Nigeria issued by the Ministry of Petroleum Resources (1991).

j. National Environmental Protection Management of Solid and Hazardous Waste Regulation (1991).

k. Establishment of the Federal Environmental Protection Agency (FEPA) Decree No 58 (1988) and Amended in Decree No 59 (1992), National Policy of the Environment (1999) (FEPA Revised Edition).

i. The elevation of FEPA to a Ministry in (1999).

(Source: Ukoli, 2005; Kadafa *et al*., 2012).

1.10. Remediation technologies for crude oil-contaminated soils

The soil is of the utmost importance to human existence and survival for numerous reasons, mostly agricultural activities (Osam *et al.*, 20011a), industrial activities and environmental sustainability. However, soil in Nigeria has been grossly exposed to contamination (Nwaugo *et al*., 2006; Osam *et al*., 2011a) through oil drilling, transportation, refining, storage and distribution of crude oil (Nogales *et al*., 2011; Chikere *et al*., 2009, 2011). Remediation of contaminated environments, particularly the soil which serves as the central point for other components of the ecosystem is very important following its contamination. There are various physico-chemical and biological methods of remediating contaminated soils that were developed over the last three decades. However, the selection of a particular method is site-specific (Khan, 2005) and these are systematically discussed. Remediation involves several measures with the fundamental objective of removing, suppressing or reducing contaminants, so that sites in their present and future use do not pose any

considerable threat to human health and the environment (European Commission, 2006 as cited in Morvan *et al.*, 2008). The contaminants released into the environment can be treated *in situ* or *ex situ*. *In situ* remediation involves treating the contaminated environment (such as soil) in its natural place while *ex situ* involves the physical removal (excavation) of soil and the contaminating substances from the polluted site to another location for treatment (Boopathy, 2000; Kapley and Purohit, 2009). In a broad sense, the various approaches used in crude oil remediation can be fashioned into physical and chemical (also referred to as engineering methods) and biological methods, besides natural attenuation (Zhu *et al*., 2004; Okoh, 2006).

1.10.1. Physical/Mechanical methods of remediation

Physical or mechanical methods are conventional approach for treating contaminated soil. This involves simple engineering methods, such as soil extraction, excavation, storage of contaminated soil as well as advanced techniques of soil electro-migration. The physical methods of remediation are expensive and often applied when the concentration of the contaminant is very high (well above compliance level) and could rely on incineration and volatilization. The cost of removing contaminants from a 1-acre of contaminated site can be estimated at 0.6-2.5 million USD (Mclntyre, 2003). It can be effective in the removal of original contaminants. Nevertheless, it is possible that the use of this method can transfer contaminants from one place to another or cause secondary pollution, as incineration residues may pose risks to the environment (Zhu *et al*., 2004; Shukla *et al*., 2011).

1.10.2. Chemical methods of remediation

Chemical methods are often applied to remediate contaminants accumulated over time in soil. This may involve modification in the physiochemical properties of such soil in order to make the soil ecologically viable and useful. The chemical methods of remediation rely on processes including extraction, pH stabilization, oxidation and reduction and precipitation. However, these are expensive and generate hazardous waste. Chemical methods also involve the use of dispersants, soil washing, soil flushing, and immobilization of contaminants as well as photochemical reduction. The physio-chemical methods of remediation are particularly expensive and not environmentally friendly (Lundstedt, 2003). It is applicable to comparatively small areas, while inapplicable to extremely contaminated soils (Khan, 2005). It often transfers contaminants from one location to another within the ecosystem (Zhu *et al*., 2004). Researchers are, therefore, exploring economically acceptable remediation technologies that will tackle the shortfalls of the physical and chemical methods of remediation.

1.10.3. Bioremediation or biological methods of remediation

In recent times, biological methods for remediation of contaminated soil have received recognition. Biological methods involve the use of organisms (plants, animals or micro-organisms); in some cases, in combination, to transform harmful substances into non-toxic ones. Bioremediation is valuable for the remediation of moderately contaminated soils (contamination below compliance level). Bioremediation involves actively aerating contaminated soil and adding fertilizer supplements to promote oil degradation by soil micro-organisms (Chorom *et al*., 2010). The organisms degrade contaminants accumulated in the soil, including their

mineralization, immobilization, or removal from the soil compartments and effectively lessen the threat of organic contaminants (Vincenza and Liliana, 2007). Microbial nitrogen (N) fixation is an attractive innovation in bioremediation of oil contaminated soils (Onwurah, 2004). Bioremediation presents remediation technology with less expertise, labour and capital requirement for oil-contaminated soil, when compared to physico-chemical methods (Obuekwe and Al-Muttawa, 2001). It can achieve complete degradation of recalcitrant organics and can be used *in situ* for pollutants, even at low concentrations (Anoliefo *et al*., 2006b). Furthermore, it is suitable for remediation of oil-contaminated soil over the traditional physico-chemical remediation methods, being more cost effective and less laborious, with the potential of less secondary environmental pollution (Kamath *et al.*, 2004). However, the efficiency of bioremediation depends on the nature and bioavailability of the soil contaminants (Rizzo *et al*., 2008).

In bioremediation, four basic biological tools are used in the remediation of oil-contaminated soil:

- Use of micro-organisms, such as bacteria and fungi to decompose organic pollutants.
- Use of living plants, particularly fast-growing species with abundant biomass, such as trees, shrubs, herbs and grasses. This is often referred to as phytoremediation.
- Use of invertebrates, such as earthworms.
- Combination of any two of the above biological techniques, or in combination with, physico-chemical methods for the treatment of oil-contaminated soils.

Remediation of oil-contaminated soil biologically has often involved the use of micro-organisms, such as bacteria or fungi, with the ability to use their enzymes to degrade pollutants in contaminated sites (Chikere *et al*, 2009; Kapley and Purohit, 2009). This method has been extensively employed in the remediation of sites polluted with organic and inorganic contaminants, because micro-organisms (such as bacterial, fungi and protozoa) use contaminants as energy sources and consequently degrade contaminants. Symbiotic N-fixing bacteria inhabiting root nodules in leguminous plants can breakdown hydrocarbons to simple compounds (Radwan *et al*., 2007; Al-Awadhi *et al.,* 2009). Various factors, such as energy and nutrient resources, microbial enzyme activity and pollutant bioavailability, determine the effectiveness of processes using of micro-organisms (Boopathy, 2000). The bioavailability of pollutants in the soil medium is determined by active transportation of pollutants and octonal water partition co-efficient in soil (Wenzel, 2009).

There are two main approaches to bioremediation, namely bio-augmentation and bio-stimulation. Bio-augmentation involves the application of suitable and beneficial microbial populations with an affinity towards a specific contaminant (Rizzo *et al*., 2008). It ensures the proper team of microbes is present in the contaminated soil in sufficient type, number and compatibility, to attack the constituents effectively and break them into their most basic compounds (Yakubu, 2007). Bio-stimulation, on the other hand, involves aeration and addition of selected micronutrients and sometimes topsoil, in appropriate quantities to stimulate the microbial community and hence promote rhizodegradation (Yakubu, 2007).

1.11. Concept of phytoremediation

Contaminants are often released into the environment (such as the atmosphere, soil and water) through human actions, such as agricultural and industrial activities, oil exploration and these substances cause environmental pollution (Ikhuoria and Okieimen, 2000; Erakhrumen, 2007; Jadia and Fulekar, 2008, 2010; Aroh *et al*., 2010). The remediation of such environments after pollution incidents is often necessary and can be effectively achieved through phytoremediation (Brandt *et al*., 2006; Erakhrumen, 2007; Nie *et al*., 2011). Phytoremediation can be defined as a process that uses the combination of plants and associated microbes, soil amendments and agronomic practices, to remove, or reduce, harmful contaminants from ecosystems (Salt *et al*., 1998; Marques *et al*., 2008). Phytoremediation is a methodology that exploits the natural ability of green plants and associated microbes to remove, degrade or suppress contaminants in soils, sludges, sediments, surface-water and ground-water, in an ecologically-friendly manner and is stimulated by sunlight. The technology is an important approach, due to its low-cost and environmentally friendly attributes (Newman and Reynolds, 2004; Kirk *et al*., 2005; Muratova *et al*., 2008; Nie *et al.*, 2011). This technology operates on the concept of using 'nature to cleanse nature', following environmental contaminationincident (Al-Awadhi *et al*., 2009; Osam *et al*., 2011b).

It is a non-destructive, cost-effective *in situ* technology that utilizes plants and their associated micro-organisms to remediate contaminated soils. Cunningham *et al.* (1996) described it as "an *in situ* use of plants and their associated micro-organisms to degrade, contain or render harmless, contaminants in soil or ground-water". The use of phytoremediation techniques can either be through naturally growing plants in

contaminated soil, or by artificial cultivation of selected plant species (Erakhrumen, 2007) and has emerged as a viable option for the remediation of petroleum hydrocarbon polluted sites (Frick *et al.,* 1999; Tanee and Kinako, 2008; Njoku *et al*., 2009). In the clean-up of contamination by petroleum hydrocarbons, plants enhance the microbial degradation of contaminants in the rhizosphere (Merkl *et al.,* 2004a, 2005a; Brandt *et al*., 2006; Atagana, 2011) and is less expensive than conventional methods (Merkl *et al*., 2004b; Rivera-Cruz, 2004).

Phytoremediation may degrade contaminants, and enhance habitat recovery, through the stimulation of plant growth. Plants can enhance bioremediation processes by absorbing, translocating or sequestering organic contaminants and removing them from the soil system (Cunningham *et al*., 1995). In a situation where the contaminant, in its present concentration, is not phyto-toxic, plant cultivation can be a valuable tool in soil remediation. The mechanism and efficiency of phytoremediation technology depends on the type of contaminant, its bioavailability and soil properties (Cunningham and Ow, 1996).

Although the phytoremediation of contaminated soil may be moderately slow, it is, however, environmentally-friendly and inexpensive, requiring little equipment and / or labour, easy to perform and has the benefit that contaminated sites can be cleaned without removing polluted soil. The key factor for successful phytoremediation practise is the identification of plant species tolerant of the contaminant and high concentrations of contaminant(s) in the polluted site. Bamidele and Agbogidi (2006) described an effective phytoremediation plant species as one that thrives well in a contaminated habitat. Some plant species of the families Poaceae, Brassicaceae,

Fabaceae, Euphorbiaceae, Asteraceae and Lamiaceae are considered as being able to remediate contaminants, due to their extensive root systems and presence of root nodules which house microbes that help degrade hydrocarbons (Jadia and Fulekar, 2008; Hall *et al.*, 2011).

Phytoremediation technology presents considerable potential for treatment of contaminated soils and has proved successful in several studies over a broad range of contaminants (Schnoor, 2002; Schröder *et al*., 2002). For instance, Merkl *et al.*, (2004b) reported that the grass: *Brachiaria brizantha* (Hochst ex A. Rich.) Stapf. and the legumes *Centrosema brasilianum* (L.) Benth. and *Calopogonium mucunoides* Desv. are good plant species for phytoremediation because in crude oil contaminated soil they combined high seedling emergence with high biomass production.

White *et al.*, (2006) investigated phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil and reported that there was enhanced degradation of complex aromatic hydrocarbons attributable to the phytoremediation process. Agbogidi *et al*. (2007) investigated the use and effectiveness of *Tectona grandis* (Linn.) and *Gmelina arborea* (Roxb.) forest tree species of family Lamiaceae for phytoremediation of crude oil contaminated soils and reported that the two plant species are good candidates for phytoremediation, especially when the concentration of the crude oil is low in the contaminated soil. Atagana (2011) reported the phytoremediation of co-contamination of crude oil and heavy metals in soil using *Chromolaena odorata* (L) King & H.E. Robins of the family Asteraceae in a pot experiment. At the end of the experiment, crude oil was decreased in the soil and attributed to natural attenuation and microbial action in rhizosphere. It was also

observed that C. *odorata* (L) has the capability of thriving and remediating crude oil contaminated soil (Anoliefo *et al*., 2006b).

Allowing polluted soil to undergo natural self-remediation takes time (Kinako, 1981). Therefore, polluted soil needs human intervention to accelerate recovery processes. The practise of phytoremediation could offer a feasible and economic alternative to achieve the remediation of petroleum hydrocarbon contaminated soils.

1.11.1. Mechanisms of phytoremediation

Remediation of soils contaminated with organic substances, including petroleum hydrocarbons, could be achieved through one, or more, of the following primary mechanisms: phyto-stabilization, phyto-extraction, phyto-degradation, phyto-volatilization, and rhizo-degradation (**Figure 1.3**) which are sub-divided on the basis of applicability and processes involved in remediating contaminated soils.

1.11.2. Phyto-stabilization

Phyto-stabilization is often referred to as the on-site activation of contaminants and is employed in the remediation of soil, sediment and sludges (USEPA, 2001; Eapen and Dsouza, 2005). In this process, plant roots limit contaminant mobility and availability within soils (Jadia and Fulekar, 2008; Mukhopadhyay and Maiti, 2010). The mechanisms involved may include absorption and accumulation by roots, adsorption onto root surfaces, or chemical precipitation within the root zone (Ghosh and Singh, 2005). Plant uptake and accumulation of petroleum hydrocarbons from contaminated soil, however, is generally limited. Thus, in the case of petroleum hydrocarbons, phyto-stabilization may simply be involved in the establishment of

vegetative cover to minimize potential migration of the contaminant through erosion, leaching or soil dispersion (Jadia and Fulekar, 2008; Raskin and Ensley, 2000). Plants (especially trees) can also act as organic pumps, transpiring water, and in turn retaining the contaminant in the root zone, thus minimizing inter-site mobility (Berti and Cunningham, 2000). Phyto-stabilization has proved successful with low concentrations (contamination below compliance level) of soil contaminants (Jadia and Fulenkar 2010). It involves accumulation of the contaminants in the root zone. The plants harbour and tolerate the contaminants within the root system and this is one of the major advantages of this process (USEPA, 2001).

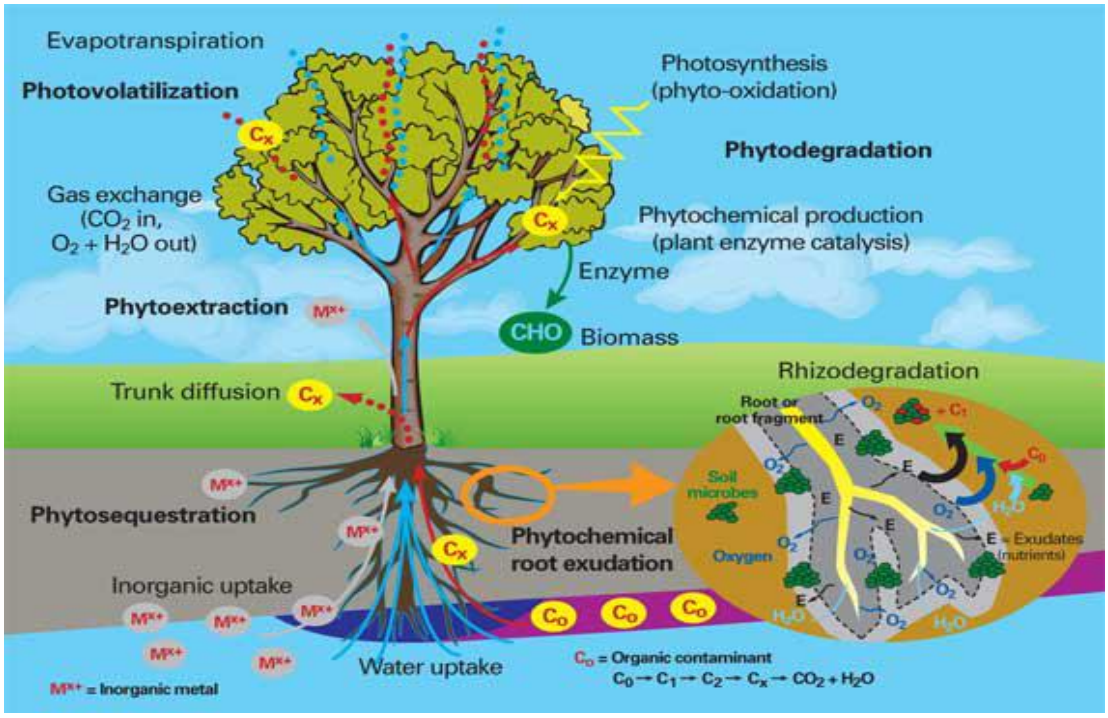


Figure 1.3: Mechanisms of phytoremediation (Source: Seslar 2005).

1.11.3. Phyto-volatilization

Phyto-volatilization refers to the use of plants for the uptake of contaminants. The contaminants are taken up by plants, converted into volatile, less chemically toxic substances and transpired into the atmosphere (Jabeen *et al.*, 2009; Jadia and

Fulekar, 2008) through the open stomata on the leaf surface and some radial diffusion from the stem tissues and plant bark (Kamath *et al.,* 2004). Some plants have the ability to absorb heavy metals (such as Selinium and mercury) and convert them to a gaseous form in plant tissues and thereafter release them into the atmosphere (Ghosh and Singh, 2005).

1.11.4. Phyto-extraction

Phyto-extraction involves the extraction of contaminants by plants through their root system and its subsequent accumulation in the harvestable aerial parts of plants (Erakhrumen, 2007). This is followed by harvesting and appropriate disposal of plant biomass. The contaminant-accumulating plants are usually cultivated by agricultural practises (Jabeen *et al.,* 2009). In the phyto-extraction process, the roots of the cultivated plant species help absorb contaminants from supporting soil, thereby reducing concentrations in the soil. With successive cropping and harvesting of plants, the concentration of soil contaminants can be decreased (Vandenhove et *al*., 2001). The cost implication of phytoextraction is greatly lower than conventional soil remediation techniques.

1.11.5. Rhizo-filtration

Rhizo-filtration relies on the capability of the plant root system to take up and sequester contaminants, or nutrients, in excess quantities from aqueous waste streams (Erakhrumen, 2007). This process has the ability to remediate metals including lead (Pb), cadmium (Cd), nickel (Ni), vanadium (V) and chromium (Cr) (Jabeen *et al.*, 2009). Plants suitable for this technique should produce extensive root systems, root biomass and surface area. The plant species should have the capability

to accumulate and tolerate substantial amounts of contaminants (Dushenkov and Kapulnik, 2000). Terrestrial plants are very appropriate for rhizofiltration. Plants such as *Helianthus annuus* (L.) of the family Asteraceae, *Brassica juncea* (L.) (Brassicaceae*)*, *Nicotiana tabacum* (L.) (Salicaceae*)*, *Spinacia oleracea* (L.) (Amaranthaceae*)* and *Zea mays* (L.) (Poaceae) have been investigated for their suitability to remove pollutants (Raskin and Ensley, 2000). Rhizo-filtration can also be conducted both *in situ* and *ex situ* to remediate contaminated water bodies. Dushenkov *et al.* (1995) recommended its commercialization and public acceptance for phytoremediation.

1.11.6. **Phyto-degradation** (sometimes referred to as **phyto-transformation**)

Phyto-degradation involves the breakdown of contaminants, either internally, through metabolic processes, or externally, through the release of plant-produced enzymes into the soil using the relationship between plants and their associated micro-organisms in the rhizosphere (Jabeen *et al.,* 2009). This can be achieved by addition of nutrients or aeration (Rezek *et al*., 2009). Some plants are capable of detoxifying contaminants and transforming them into non-phytotoxic metabolites. These contaminants are detoxified in three phases: conversion, conjugation and compartmentalization (Kamath *et al.,* 2004). Phyto-degradation relies on plant enzymes to metabolize, or mineralize contaminants (Jabeen *et al*., 2009). Plants and micro-organisms are involved, both directly and indirectly, in the degradation, or transformation of petroleum hydrocarbons into products that are generally less toxic and persistent in the environment than the parent compounds. Phyto-degradation usually occurs in the rhizosphere.

1.11.7. Rhizo-degradation or rhizo-remediation

Rhizo-degradation, otherwise referred to as rhizo-remediation, is applied in the remediation of pollutants, such as petroleum hydrocarbon contaminated soils. This process involves the use of tolerant plant species, and associated micro-organisms, in the rhizosphere to accelerate remediation processes (Pajuelo *et al*., 2011). Symbiotic N-fixing bacteria inhabit the root nodules of leguminous plants and can be used to breakdown complex hydrocarbons to simple compounds (Radwan *et al*., 2007). Plant root systems suitable for rhizo-degradation support adequate microbial growth due to their ability to offer their root nodules as a habitat (for microbes, enzymes, nutrients and oxygen) as well as a large surface area for microbes to colonize soil layers (Anderson *et al*., 1993). Roots are capable of releasing 'degradative enzymes' to promote degradation of petroleum hydrocarbons (Wenzel, 2009). Root systems also play significant roles in transferring contaminants to the degrading microbes and for oxygenation, either by transferring O_2 or creating a vacuum in the soil sub-surface that permits diffusion of atmospheric O_2 (Van Epps, 2006).

In the rhizosphere a much higher microbial density (which could enhance rhizo-degradation) is present in surface soils than in deeper layers (Hinsinger *et al.,* 2005, 2006) and this is associated with higher microbial numbers, diversity and bioactivity (Boopathy, 2000). Availability of numerous degrading microbes in the soil significantly determines their potential for remediation (Mikkonen *et al.,* 2011). Bacteria in the soil rhizosphere are increased by organic contaminants (Chaineau *et al.,* 2003; Chaudhary *et al*., 2012). This increased microbial population, and its availability, promote plant growth through the degradation of organic contaminants. The *Rhizobium* spp. population helped increase the growth performance of *Trifoliun* spp. (L.) Fabaceae on hydrocarbon contaminated soil (Chiapusio *et al.,* 2007).

Rhizo-remediation can be employed in the treatment of soil contaminated by petroleum hydrocarbons, but the choice and tolerance of plant species also influence its effectiveness.

1.12. Tolerance mechanisms of plants and suitability for remediation

Physiological and molecular mechanisms determine the suitability of plants species for remediation processes. A plant's tolerance to a particular contaminant is governed by its ability to tolerate an increasing level of contaminant (Jabeen *et al*., 2009). Kamath *et al.* (2004) identified some criteria for selecting plant species. This should follow the needs of the application, the contaminants concerned and the potential of such species to thrive well on contaminated soil. It is preferable to use native plant species for remediation purposes to support soil ecosystem restoration (Pilon-Smits and Freeman, 2006), as introduced, or exotic species, may become invasive during, or after, the clean-up exercise thus causing other ecological problems.

1.13. Selection of plant species for phytoremediation

Researchers have investigated the selection of plants for the remediation of hydrocarbon contaminated soils (Merkl *et al.,* 2004a; White *et al.*, 2006; Agbogidi *et al*., 2007; Atagana, 2011). There was enhanced degradation of complex hydrocarbons within the root rhizosphere (Merkl *et al.,* 2004b; Atagana, 2011). This suggests that a good plant candidate for phytoremediation must have an extensive root system and the plant, with its associated microbes, must be able to survive and grow in the crude oil-contaminated soil (White *et al*., 2006) and must be fast growing (Brandt *et al*., 2006). The use of perennial plant species is considered more suitable than annual species. The moisture content and the climatic factors also

influence the decision on plant selection for phytoremediation (White *et al*., 2006). Plants used for phytoremediation should be adaptable to the climatic and soil conditions prevailing in the contaminated sites and such plants should be able to withstand stress.

The selection of suitable plant species is a fundamental step to be considered in phytoremediation processes. Some plants do not tolerate the presence of contamination, while others do and effectively enhance remediation. This may be due to variation in plant morphology (e.g. roots), physiology and biochemistry (e.g. root exudates) and interactions between microbes and the plants in the rhizosphere (Walker *et al.,* 2003). Some grasses, herbs, shrubs and trees are good candidates for phytoremediation (**Table 1.5**) and some of these plants have extensive branched fibrous roots that are more likely to provide large surface areas for interaction (Yateem *et al.,* 2007). The rhizospheres of certain trees (e.g. *Populus deltoides x nigra*) have the capability to enrich hydrocarbon degrading micro-organisms more than soil outside the root zone (Hutchinson *et al.,* 2003). To achieve maximum hydrocarbon remediation and to successfully establish a stable vegetation cover, various criteria must be considered. Any ideal plant species candidate should be selected to provide a large root surface area per unit volume of soil (Aprill and Sims 1990; Smith *et al.,* 2006), which thus promotes rhizosphere-contaminant-microbe interactions. Due to the frequent poor nutrient availability in contaminated sites (Kirkpatrick *et al.,* 2006; Wenzel, 2009) they should be able to tolerate and thrive with low N and phosphorus (P) availability.

Table 1.5: Plant species with demonstrated potential to phytoremediate petroleum hydrocarbons

Common name	Scientific name	Family	Source
Alfalfa	*Medicago sativa* L.	Fabaceae	Nichols *et al.* (1997)
Alpine blue grass	*Poa alpina* L.	Poaceae	Nichols *et al.* (1997)
Bermuda grass	*Cynodon dactylon* (L.) Pers.	Poaceae	Reynolds *et al.* (1999)
Bush bean	*Phaseolus vulgaris* L.	Fabaceae	Frick *et al*. (1999)
Carpet grass	*Axonopus compressus* (Sw.) P.Beauv	Poaceae	Efe and Aboh (2012); Efe and Elenwo (2014)
Cow pea	*Vigna unguiculata* L.	Fabaceae	Tanee and Kinako (2008)
Tall Fescue	*Festuca arundinacea* Schreb	Poaceae	Reynolds *et al*. (1999); Dzantor *et al.* (2000)
Little bluestem	*Schizachyrium scoparium* (Michx.) Nash	Poaceae	Pradham *et al.* (1998)
Miracle tree	*Leucaena leucocephala* (Lam.) de Wit	Fabaceae	Osam *et al.* (2011a)
Nut sedge	*Cyperus rotundus* L.	Cyperaceae	Efe and Aboh (2012)
Eastern cottonwood	*Populus deltoides x nigra* L.	Salicaceae	Frick *et al*. (1999)
Rattle weed	*Crotalaria retusa* L.	Fabaceae	Osam *et al.* (2011a)
Italian Rye-grass	*Lolium multiflorum* Lam.	Poaceae	White *et al.* (2006)

Sorghum	*Sorghum bicolour* (L.) Moench	Poaceae	Frick *et al*. (1999)
Maize	*Zea mays* L.	Poaceae	Liao *et al*. (2015)
Soybeans	*Glycine max* Willd	Fabaceae	Njoku *et al*. (2009)
Sudan grass	*Sorghum vulgare* (L.) Moench	Poaceae	Frick *et al*. (1999)
Switch grass	*Panicum virgatum* L.	Poaceae	Pradham *et al.* (1998)
Vetiver	*Vetiveria zizanioides* (L.) Nash	Poaceae	Brandt *et al*. (2006)
Yellow flame tree	*Peltophorum pterocarpum* (DC.) K. Heyne	Fabaceae	Osam *et al.* (2011a)
Bambara groundnut	*Vigna subterranea* (L.)	Fabaceae	Nwaichi *et al*. (2010).

1.14. Suitability of Fabaceae for phytoremediation of hydrocarbon contaminated soils

The Fabaceae family is made up of plant species commonly referred to as legumes and consists of ~18,000 species across the world and grow in diverse terrestrial habitats. The potential and suitability of Fabaceae for phytoremediation of hydrocarbon polluted soil, with its unique adaptation and rhizodegradation mechanisms, is well known (Merkl *et al.,* 2004b; Tanee and Akonye 2009; Atagana, 2011; Osam *et al*., 2011b; Hall *et al.*, 2011). There are several reports on the use of legumes in hydrocarbon contaminated soil remediation and their ability to fix N (Nichols *et al.*, 1997; Dzantor *et al.,* 2000; Osam *et al*., 2011b).

Contaminated soils are usually particularly deficient in N and P (Wenzel 2009) and competition for nutrients among soil biota decrease nutrient availability. N fixing plant species, such as legumes, can be used in rhizoremediation (Miller and Cramer, 2004). Microbes, such as *Rhizobium* species, can penetrate the root systems of leguminous plant species and form symbiotic interactions in their root nodules, with which they are able to fix atmospheric N in the form of ammonium compounds (Suominen *et al.,* 2000) and have also been found to increase potassium (K) and P uptake in plants (Vershinina, 2012). Some of the common N-fixing microbes in soil include *Azotobacter* spp., *Azospirillum brasilense*, *Rhizobium* spp. and Actinomycetes (Havlin *et al.,* 2005) and these micro-organisms play vital roles in remediation work by degrading contaminants. The amount of N fixation by microbes in plant root nodules is substantial, often >100 kg ha^{-1} y^{-1} (Vitosek *et al*., 2002). The interaction between microbes and leguminous plant species, such as alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*), have proved successful in the remediation of petroleum hydrocarbon contaminants (Frick *et al.*, 1999). The use of

woody leguminous plant species for phytoremediation in tropical areas is a reflection of their prevalence and abundance (Vitosek *et al.,* 2002) and they can stimulate microbial growth, which increases oxidation of organic compounds (Peer *et al.,* 2006).

1.15. Comparison of phytoremediation with alternative remediation strategies

Phytoremediation has shown remarkable cost effectiveness and recent societal acceptance. Its advantages include low costs (Frick *et al.,* 1999; Macek *et al.,* 2000; Glick 2003). Other advantages compared with other remediation processes include:

- Can be applied *in situ*.
- Offers less disruption to the natural environment as compared with mechanical methods.
- Avoids excavation and damage to soils.
- Can be applied to large areas of terrestrial contamination.
- Relatively easy to apply.
- Preserves and enhances soil structure.
- Potentially quick to apply to the contaminated sites.
- No disposal site(s) is required.
- Can be applied to a diverse range of hazardous materials.
- Plants act as indicators of contamination.
- Plants help contain contaminants.
- Plants transfer oxygen and nutrients to the rhizosphere.

- Other additional advantages of providing plant cover (e.g. erosion control, reduced leaching, landscape aesthetics, improved habitat for fauna and carbon-sequestration (Frick *et al.*, 1999).

The success of previous phytoremediation works, using a range of plant species has shown that research on this emerging technology should be encouraged, strengthened and applied where applicable (Nichols *et al.*, 1997; Dzantor *et al.*, 2000; Tesar *et al.*, 2002; Merkl *et al.*, 2004a; Bamidele and Agbogidi, 2006; Atagana, 2011). This is especially the case in areas prone to hydrocarbon contamination such as the Niger Delta region of Nigeria.

1.16. General overview of soil-amendments in the remediation of contaminated soils

This section reviews the potential soil amendments in the remediation of contaminated soil. It provides information on the use of fertilizers and selected natural zeolites such as clinoptilolite and kaolinite as useful soil-amendments. Increased crude oil exploration has accelerated soil contamination due to oil spillage, particularly in the nations with large oil resources, such as Nigeria. Many materials for oil remediation have been used in these oil producing nations for partial or total remediation of the soil.

Organic and in-organic fertilizers and natural zeolites play vital roles in phytoremediation processes. Fertilization is important in phytoremediation protocols (Merkl *et al.*, 2005c; White *et al.*, 2003, 2006; Tanee and Kinako, 2008). The addition of fertilizers (as a soil amendment) and periodic tillage are useful in the degradation of petroleum hydrocarbons in contaminated soil Chaineau *et al.* (2003).

However, excessive use of N-fertilizer can damage the environment and to avoid this problem, N-fixing plant species supplemented with soil amendments are encouraged in remediation (Miller and Cramer, 2004). The environmental applications of natural zeolites as a viable soil amendment has been reported for contaminated soils (Ming and Allen, 2001; Bowman, 2003; Chmielewska, 2003; Tian *et al*., 2004; Englert and Rubio, 2005; Leggo *et al.,* 2006; Oguz *et al*., 2010; Misaelides, 2011) and for the restoration of soil nutritional qualities. Kelay *et al*., (2015) affirmed the effectiveness of Na-chabazite and the potential of zeolites for low-cost remediation by adsorption of oil from contaminated soils. Adebowale *et al.*, (2005) reported the environmental significance of Nigerian kaolinite for adsorption and remediation of Pb^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} metal ions in soil media. Trckova *et al.*, (2004) affirmed that kaolinite is effective in the amelioration of adverse effects of contaminants.

1.16.1. The use of fertilizer as a soil amendment in contaminated soil remediation

Contaminated soils are often deficient in nutrients, particularly N and P (Wenzel, 2009). This may be due to high competition for available nutrients in the soil. Under extreme conditions, especially when soil temperature or moisture content is low, N deficiency is intensified due to poor nutrient transportation and restricted enzyme and microbial activities (Wenzel, 2009). In an effort to resolve this problem, some studies suggested the use of fertilizer-phytoremediation (Parrish *et al*., 2004). Adequate fertilization and periodical tillage are helpful in petroleum hydrocarbon (PHCs) degradation (Chaineau *et al.,* 2003). Fertilization of crude oil contaminated soils improved nutrient status particularly N and P with adequate degradation of petroleum hydrocarbons at the rhizosphere (Unterbrunner *et al*., 2007). Bio-

stimulation and phytoremediation of tropical soil of the Niger delta of Nigeria found most crude oil biodegradation and improvement in soil nutrient content was observed in NPK 15:15:15 treated samples (Tanee and Kinako, 2008). Fertilizers proved useful in bioremediation processes, as agricultural fertilizers (NPK) enhanced the microbial degradation of petroleum hydrocarbons (White *et al*., 2003; Brandt *et al.,* 2006; Chorom *et al.,* 2010). A N-fixing bacteria assisted cropping system is therefore ideal for the successful remediation of contaminated soils if enhanced with proper management strategies, such as irrigation, fertilization, weed control (mowing, mulching, or spraying) and pest control (ITRC 2009).

1.16.2. Zeolites as suitable amendments of contaminated soils

Zeolites are stable three-dimensional honeycomb crystalline, alumina-silicate materials with micro-porous hydrated structures. They have immense academic, scientific and industrial interests in the areas of ion exchange (detergent industry, radioactive waste storage, and treatment of liquid waste), separation (purification, drying, environmental treatment) and petroleum refining along with petrochemical, coal and fine chemical industries (Breck, 1974; Chiang and Chao, 2001; Xu *et al*., 2007). Zeolites have AlO_4 and SiO_4 tetrahedra that are linked together by sharing all the oxygen atoms to form interconnected cages and channels containing movable water molecules and alkali metals e.g. Na^+, Ca^+ and K^+ (Englert and Rubio, 2005; Sahner *et al*., 2008). Zeolite minerals are used in processes such as catalysis, molecular sieving, refining, ion exchange and environmental protection and management (Bebon *et al*., 2002; Caballero *et al*., 2007; Ajayi *et al*., 2012). The ability of zeolites to act as multi-functional materials in many industrial applications

is due to their inherent properties, such as uniform pore size/shape, mobile cation and hydrophilicity/hydrophobicity (Berendsen *et al*., 2006).

1.16.2.1. Occurrence of zeolites

Zeolites occur in natural and synthetic forms. Naturally occurring zeolites, such as clinoptilolite, mordenite and chabazite are generally cheap and abundant (Weitkamp, 2000; Wang *et al.,* 2006; Wang and Zhu, 2006). Over 50 natural zeolites have been discovered, with seven of them in large deposits: analcime, chabazite, clinoptilolite, heulandite, natrolite, phillipsite and stilbite (Bogdanov, 2009). Natural zeolites are usually volcanic products, but synthetic zeolites are produced through chemical reactions usually in laboratories. These forms of zeolites are gaining wide acceptance and becoming essential due to their numerous physico-chemical properties (Wang *et al.,* 2006). Zeolites are valuable and widely used adsorbent agents, due to their high ion exchange, and adsorption properties and thermal stability (Alpat *et al*., 2008).

1.16.2.2. Classification and Nomenclature of zeolites

Zeolites are classified based on their crystal structure and chemical composition (Szostak, 1989). The first structural classification of zeolites is based on the term ‘framework topology’ and in this classification scheme a particular framework receives a three-letter code (Ertl *et al.,* 2008). The second structural classification of zeolites is based on the concept of ‘secondary building unit’ (SBU), which involves the geometric arrangement of tetrahedra structure (Breck, 1974). The third structural classification scheme operates on an historical context, such as discovery and naming of the zeolites, in addition to the terms discussed in the second scheme above. There is no particular systematic nomenclature for zeolites. A unique zeolitic

structure which has been established with a particular structural type is assigned a name and three-letter code (LTA for Linde zeolite A, MFI for zeolite ZSM-5) by the Structure Commission of the International Zeolites Association (IZA) (**Figure 1.4**).

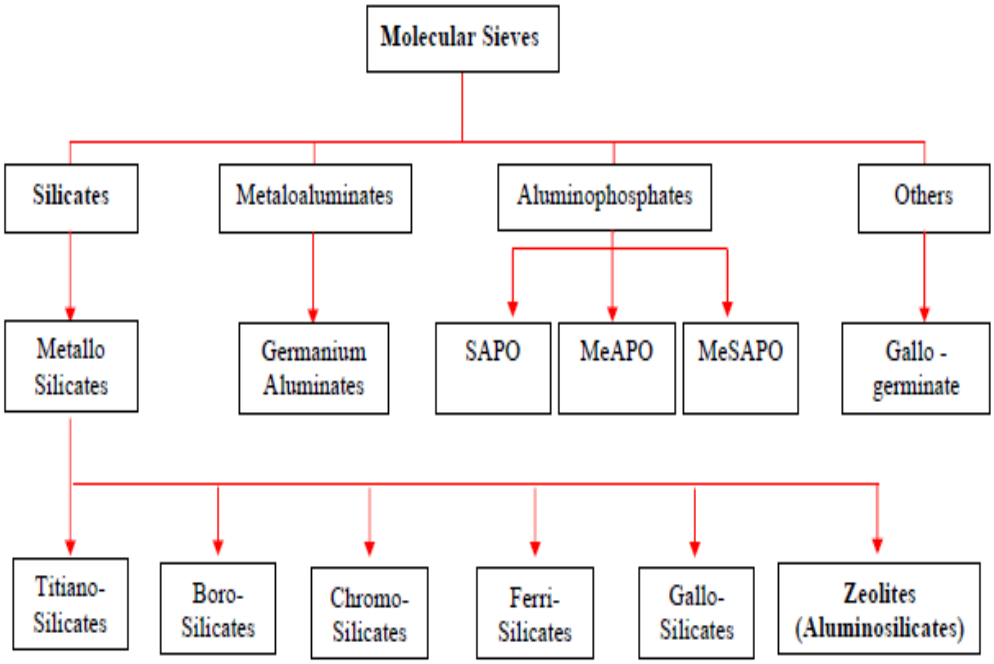


Figure 1.4: Classification of molecular sieve materials (Source: Jakkula, 2006).

1.16.2.3. Structure of zeolites

Zeolites have a very porous structure (Kyotani *et al*., 1997; Bogdanov *et al*., 2009) which allows the passage of some ions and the blockage of others (Alvarez *et al.*, 1995). Cations freely move in and out of the framework structure and such movement allows ion exchange (**Figure 1.5**). Tetrahedra are linked together to form cages connected by pore openings of definite sizes, ranging from 0.3-1 nm (Szostak, 1989).

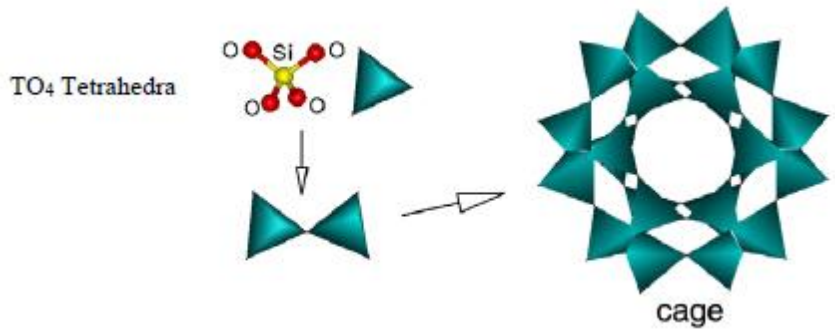


Figure 1.5: Tetrahedral framework showing cage structure of a given zeolite (Source: http://www.bza.org/zeolites.html).

Zeolite minerals are often represented chemically with a empirical formula, as postulated by (Barrer, 1982):

$$(M_x^+, M_y^{2+})[Al_{(x+2y)}Si_{n-(x+2y)}O_{2n}].mH_2O \qquad 1(1)$$

Where:

Atoms in the tectosilicate framework structure are represented within parentheses.

The cations (monovalent and divalent) that neutralize the structural negative charge are represented within parentheses by M^+ and M^{2+}.

Water molecules represent absorbed water.

The total number of tetrahedral cations (Al + Si) in a unit cell is n.

The number of oxygen atom is 2n.

1.16.2.4. Pores and channels of zeolites

The pore opening and dimension of channel system are often used to describe the structural framework of zeolites (Szostak, 1989). These pore openings are characterized by the size of the ring which defines a given pore, usually designated as an n-ring, where n is the number of T-atoms in a ring. Different pore openings are given different ring sizes. An 8-ring is regarded as a small ring, a 10-ring a medium

ring opening and a 12-ring is a large ring opening. Eight-ring zeolites are good catalysts for some small scale reactions, but the 10- and 12-ring ones are usually preferred catalysts for various reactions (Weitkamp *et al.*, 2001). Different methods that have been proposed to examine pore size, but the simplest and most commonly used one involves selecting the proper molecular probes and investigating the capability of the zeolite to adsorb these probes. The size and shape of the pore opening depend on several factors (Szostak, 1989), including:

- Configuration of the T and O atoms relative to each other.
- Size of the action.
- Silicon/Aluminum ratio.
- Location of the cation.
- Temperature.

Some zeolites and their micro-pores are as shown in **Figure 1.6**.

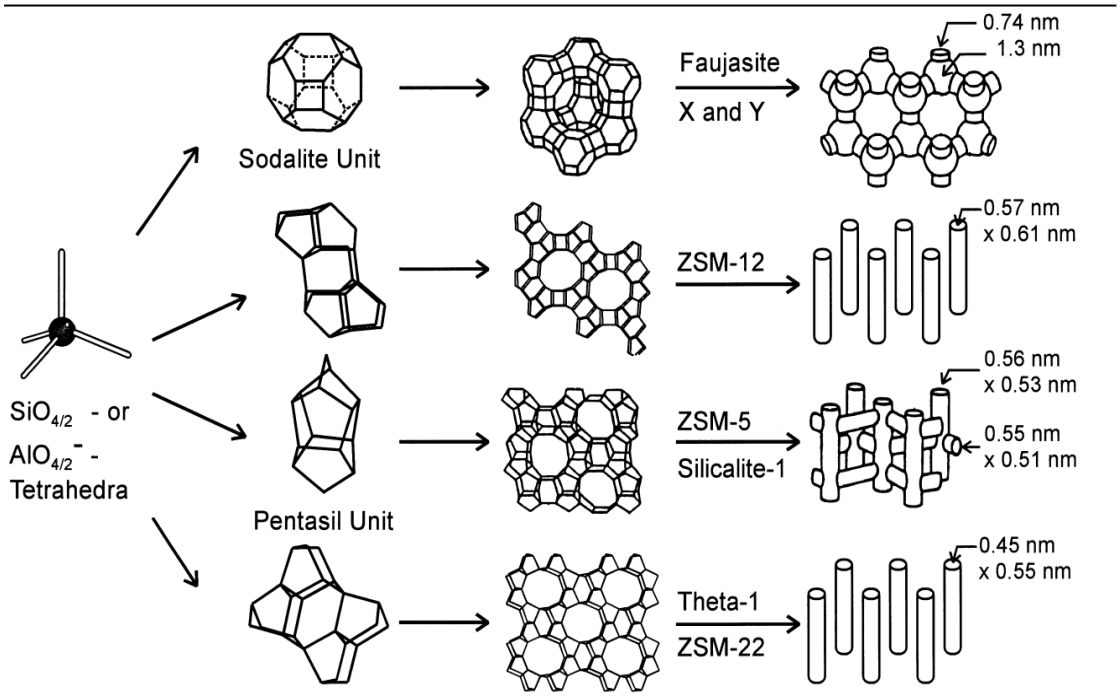


Figure 1.6: Structure of some zeolites (from top to bottom: Faujasite or zeolites X, Y; zeolite ZSM-12; zeolite ZSM-5 or silicalite-1; zeolite Theta-1 or ZSM-22) and their micro-pore systems and dimensions (Source: Weitkamp, 2000).

It is important to consider the channel system within a given zeolite framework with the pore size. Zeolites can be considered as 1-, 2- or 3-dimensional tubes or channels rather than as 8-, 10- and 12-ring pore openings. In some zeolites, the tubes or channels are very short. Examples of some zeolites and their channel systems are shown in **Table 1.6**.

Table 1.6: Selected zeolites and their channel systems

Channel System	Zeolites
One-dimensional	Analcime
Two-dimensional	Mordenite, Phillipsite
Three-dimensional	Paulingite, ZSM-5, ZSM-11

(Source: Jakkula, 2006).

1.16.2.5. Secondary Building Unit (SBU)

The tetrahedra framework of AlO_4 or SiO_4 represents the primary building units. The alumina and silica tetrahedral frameworks are combined into more complex secondary units which form building blocks of the zeolite crystal structure. Secondary building units (SBU) consist of selected geometric groupings of those tetrahedra (Borade and Clearfield, 1997). All zeolite structures are expressed using the building units. The various secondary building units recognized in zeolite frameworks are presented in **Figure 1.7**.

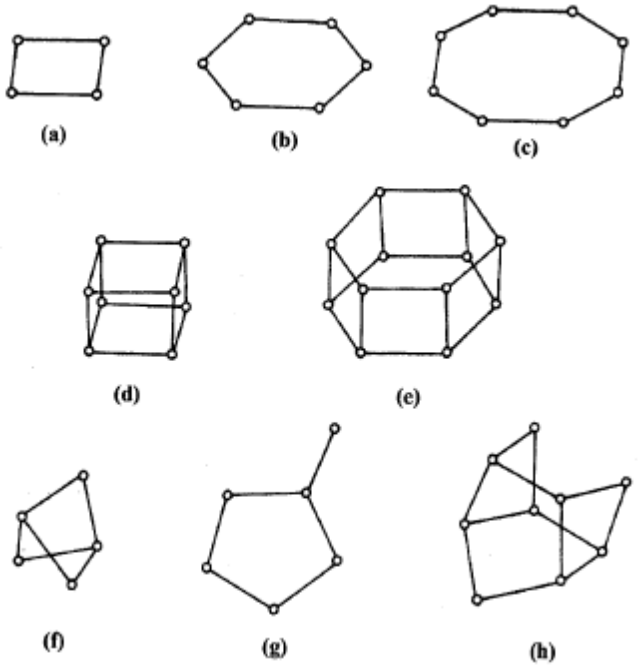


Figure 1.7: Secondary building units recognized in zeolite frameworks (a) Single four ring (S4R), (b) Single six ring (S6R), (c) Single eight ring (S8R), (d) Double four ring (D4R), (e) Double six ring (D6R), (f) Complex 4-1, (g) Complex 5-1 and (h) Complex 4-4-1 (Source: Jakkula, 2006).

Most zeolite frameworks are derived from various different secondary building units. The existing similarities and differences in zeolite frameworks thus require a building unit that considers the arrangement of these secondary building units in space. Two rings can also join together to form more complex and extended chain building units (Szostak, 1989). An extended zeolite structure can also be described in the form of two-dimensional sheet units. It is simple to compare adsorption and catalytical abilities of zeolites using their openings and channel systems.

1.16.2.6. Properties of zeolites

Zeolites exhibit many physico-chemical properties, including:

- Particle size.
- Morphology.
- High cation hydrolysis/exchange capacities.

- Hardness.
- Thermal expansion.
- Density.
- Dehydration.
- Stabilization.
- Cation selectivity.
- Molecular sieving.

These physico-chemical properties of zeolites make them suitable for agricultural activities (Barbarick and Pirela, 1984; Allen and Ming, 1993, Jakkula *et al*., 2006) and as soil remediating agents for environmental protection (Ming and Allen, 2001). Zeolites are used in agriculture as soil amendments and some of the relevant qualities include: decreased soil acidity; activating nutrients from soil reserves and decreasing the need for mineral fertilizers. In turns, this eliminates fertilizer acidifying effects; toxic effects and increasing drought resistance by binding water molecules (Pisarovic *et al*., 2003). The high cation-exchange capacities, cation selectivity and molecular sieving abilities make them suitable for contaminated soil remediation (Ming and Allen, 2001).

1.16.2.7. Applications of natural zeolites

Many potential applications have been identified for zeolites (Metes *et al*., 2004). Zeolites are useful in processes including ion exchange, filtration process, odour control, water softening and adsorption processes, catalysis, soil stabilization and conditioning, soil amendment, slow-release fertilizers, soil-less substrates, carriers for insecticides and pesticides, water treatment, paint components with anti-corrosive properties, fixation of phosphates, clean-up of sewage, ammonium ion removal and

as remediation agents in contaminated soils (Ming and Allen, 2001; Polat *et al.*, 2004; Reháková *et al*., 2004; Terzano *et al*., 2005; Jakkula *et al*., 2006; Beqiraj *et al*., 2008; Milošević and Milošević, 2010). Natural zeolites such as clinopileolite (Obua *et al*., 2014), Na-chabazite (Kelay *et al*., 2015), Kaolinite (Oyedeji *et al*., 2015a) have been investigated as partial solutions to environmental contamination with oil. Further review on kaolinite is presented in Section **1.17**. These natural zeolites are applicable to environmental contamination remediation owing largely to their plasticity and high sorption capacity (**Figure 1.8**).

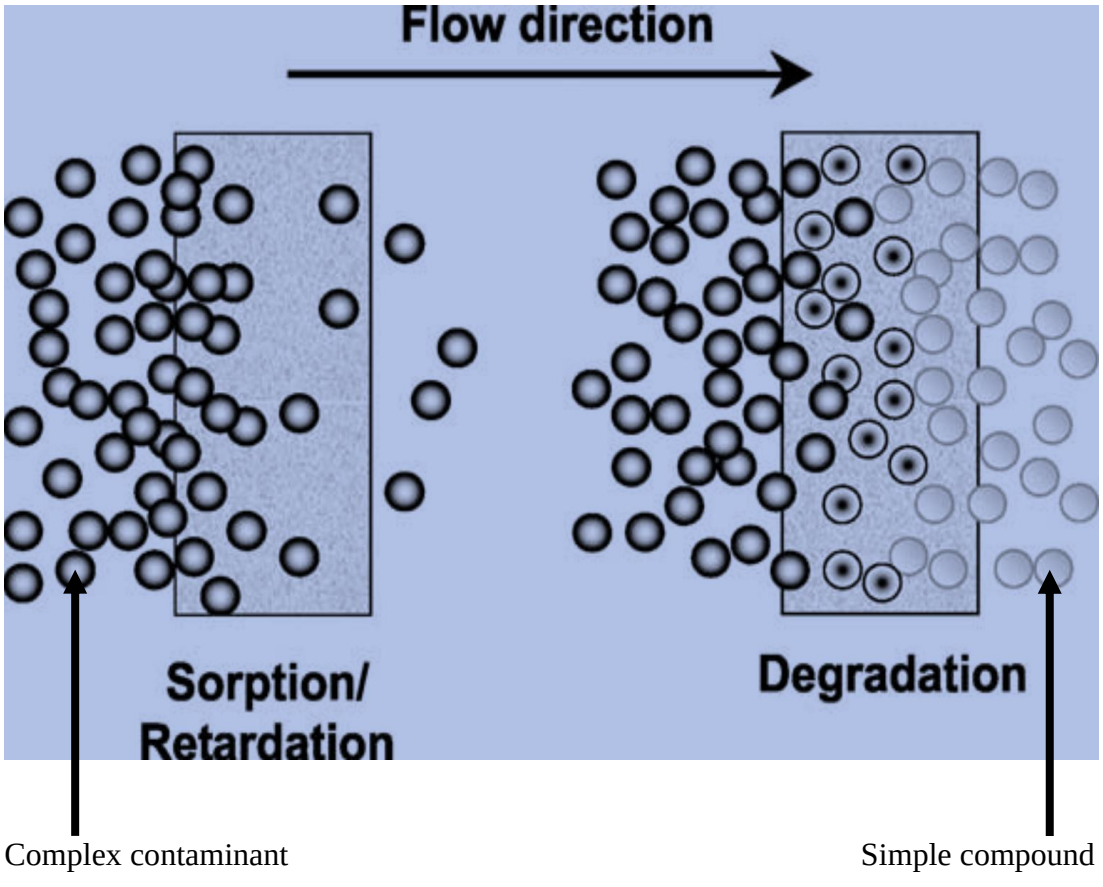


Figure 1.8: Schematic diagram showing sorption reaction in natural zeolites (Modified after: Bowman, 2003).

1.17. An overview of kaolinite and its deposition in Nigeria

Kaolin is the primary clay mineral material present in the kaolinite mineral group (Brigatti *et al.*, 2006). The name kaolin was coined from the Chinese word called 'Kao-Ling' meaning 'Highhill' (Bergaya and Lagaly, 2006). More importantly kaolin is viewed industrially as a term that means clays composed mainly of kaolinite and is amenable to property variation, making them useful in the production of industrial products (Murray, 1980). Impurities such as quartz, feldspar, and iron are usually found with clay minerals but because they do not exhibit plasticity, they are called non-clay or accessory minerals (Bergaya and Lagaly, 2006). This associated mineral requires removal or decrease because it generally reduces the commercial value of clay minerals, hence purification is very important before use.

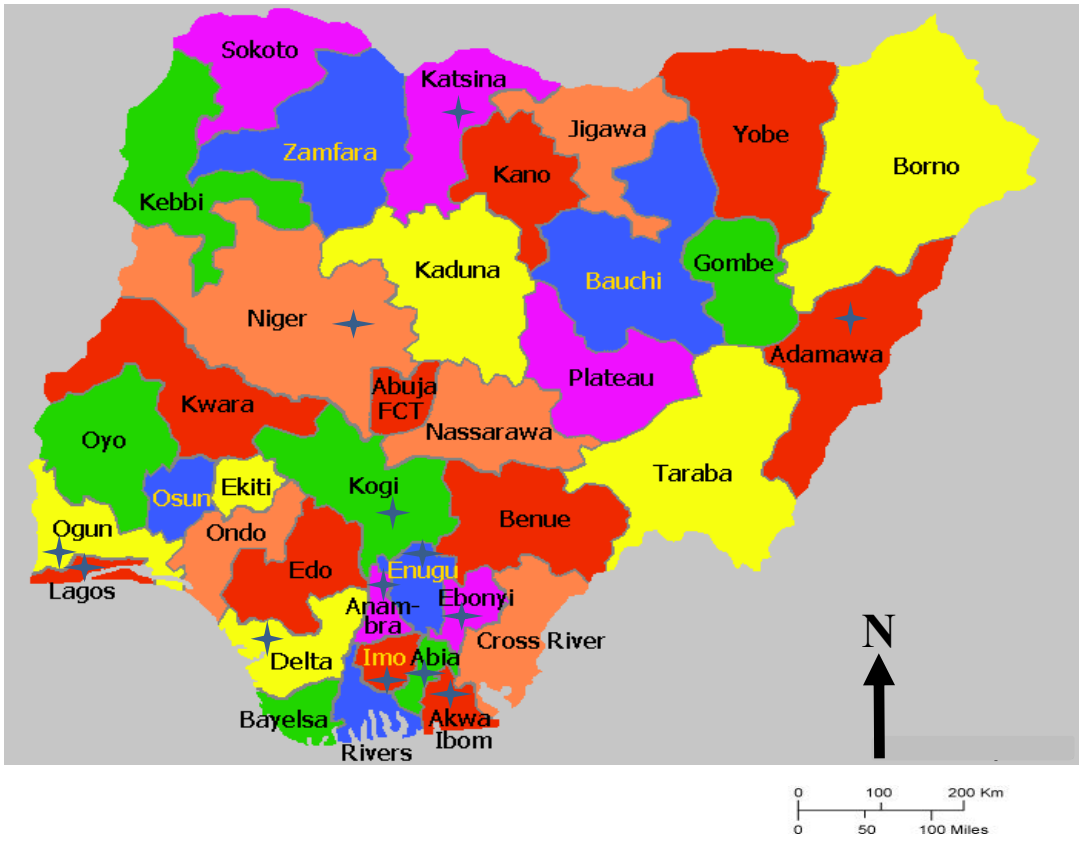


Figure 1.9: Map of Nigeria showing selected states with kaolinite resources (✦ **)**

1.17.1. Structure of kaolinite

Kaolin is a layered plastic raw material which consists primarily of the clay mineral kaolinite and has the chemical formula: $Al_2O_3.2SiO_2.2H_2O$ (39.5% Al_2O_3, 46.5% SiO_2, 14.0% H_2O) (Trckova *et al*., 2004). It can be viewed as a continuous two dimensional structure containing a silicatetrahedral sheet with a central cation, usually octahedral alumina, which is linked to four shared oxygen atoms (**Figure 1.10**).

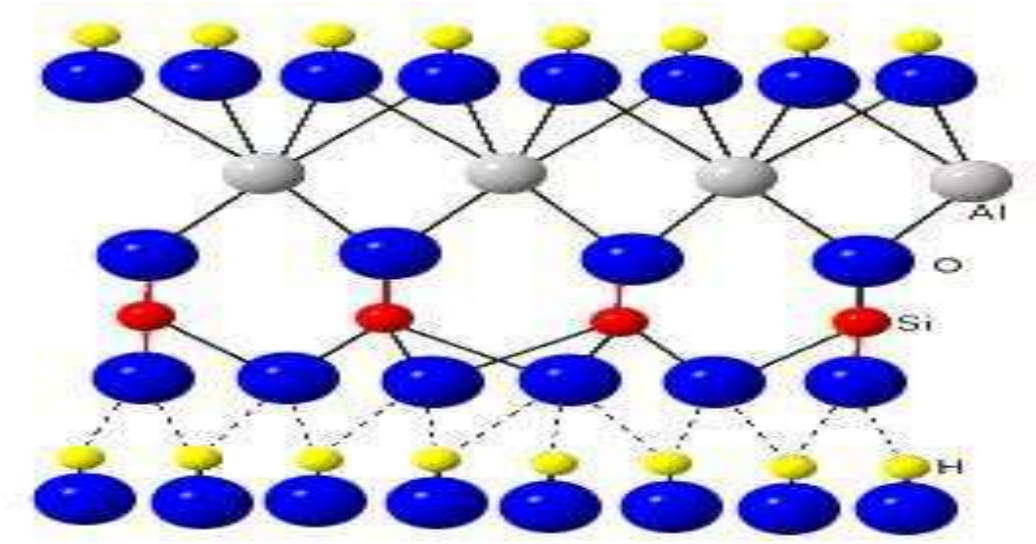


Figure 1.10: The layered structure of kaolinite (Source: Kovo, 2011).

The position of Si, Al and O in the kaolinite structure is well documented, but the location of the OH is in doubt. However, the bonding system of OH in the interlayer has been explained by Benco *et al.* (2001). Four different OH groups were identified and two are part of weak bond formation and the remainder do not participate in H-bonding. The surface of clay minerals is usually hydrophobic. However, in kaolinite, the presence of the hydroxyl group and defect sites at the surface introduces hydrophilicity. The defects present can easily be detected with the aid of XRD (Kovo, 2009). The patterns of ordered kaolinite are sharp and narrow in their peaks,

while disordered show broad and asymmetric peaks (Bergaya and Lagaly, 2006). Two tests are normally performed to identify the degree of order in a kaolinite sample, namely the Hinckley Index Range and Weiss Index (Bergaya and Lagaly, 2006).

Kaolinite obtained naturally is usually fractionated to enrich the kaolinite content and decrease unwanted clay minerals before application in manufacturing materials, such as zeolites (Chipera and Bish, 2001; Elton *et al.*, 1992). The most common and simplest method of enriching the kaolinite content of raw kaolinite is fractionation by sedimentation (Bergaya and Lagaly, 2006). The refining process of kaolinite is clearly divided into two groups namely removal of foreign material by chemical methods and refinement by sedimentation to remove larger impurities, especially quartz, which is trapped within the mineral aggregates. However, addition of chemicals in the treatment process can impair the properties of the parent material, therefore, the use of chemical treatment is usually minimized (Chipera and Bish, 2001).

Even though there are several other methods, such as selective flocculation, flotation, delamination and ultrasonic treatment that can be used to process raw kaolinite, fractionation by sedimentation is the most common procedure used for kaolinite processing to obtain highly pure laboratory grade kaolinite (Chipera and Bish, 2001). Sedimentation is based on the principle that a particle with different mass and density will settle at different terminal velocity in a given viscous media (Pabst, 2000). The largest usage of kaolinite is in the paper industry, where it is utilized as a filler and coating agent. Other uses of kaolinite include: additive in paint production,

production of ceramics, sanitary ware and electrical porcelain (Ekosse, 2010). There is growing interest in the use of kaolinite as a combined source of silica and alumina for zeolite synthesis (Xu *et al*., 2007). The use of different kaolinite deposits around the globe to synthesize zeolites has gained ground.

Nigeria has ~3 billion tonnes of kaolinite clays which occur in deposits of greatly varying nature and spread across many states of the country (Kovo, 2011). The available clays in the different deposits have a great deal of differences and frequencies (Fakolujo *et al*., 2012). Notable among the Nigerian kaolinite deposits are Kankara in Kastina State (Atta *et al*., 2007; Ajayi *et al*., 2012), Ahoko in Kogi State (Kovo, 2011), Ukpor in Anambra State (Igbokwe *et* al., 2008), Ovwian in Delta State (Oghenejoboh and Ohimor, 2011), Ubulu-Ukwu in Delta State (Adebowale *et al*., 2005; Ekosse, 2010) and Abeokuta deposit in Ogun State (Fakolujo *et al*., 2012). Large kaolinite deposits occur in the Niger Delta region. The mineralogical characterization of clay soil samples from south-western Nigeria showed that kaolinite as the primary mineral (Fakolujo *et al*., 2012) showing the wide spread distribution of kaolinite in Nigeria (**Figure 1.9**). Currently, there are two kaolinite processing plants in Nigeria, Kankara kaolinite factory in Katsina State, and Crystal kaolinite factory in Plateau State. These two processing plants were established in 1998 to service the paper making industries. The applicability of kaolinite in the remediation of contaminated environments leaves more to desire and the abundance of these natural resources in Nigeria, which could be re-directed to pre-treat contaminated soil prior to phytoremediation activities.

1.18. An overview of the selected leguminous tree species (LTS)

1.18.1. *Albizia adianthifolia* (Schumach.) W.F. Wright

Albizia adianthifolia is a tree that belongs to the sub-family Mimosoideae in the family Fabaceae. It is commonly known as West African Albizzia, which is widely grown in southern Nigeria (Aigbokhan, 2014). It widely cultivated and naturalized in the sub-tropics and tropics. It serves as good source of timber (Khan and Tripathi, 1987; Nyananyo, 2006), particularly for the people of the Niger Delta region and its other potential benefits include environmental management, forage sources and medicine. Singh *et al*. (2004) found *A. lebbeck*, which belongs to the same family as *A. adianthifolia* tolerant and efficient in the decreasing heavy metal concentration in the soil of a mining site and for vegetation re-establishment.

1.18.2. *Albizia odoratissima* (Linn.) Benth.

Albizia odoratissima belongs to the Fabaceae family. It is a fast-growing, deciduous tree reaching 15-25 m height with a trunk diameter of 120-150 cm. It is one of the top N-fixing tree species growing in forested zones. It grows well in a wide range of habitats with an extensive distribution in the tropics, particularly tropical Africa. *A. odoratissima* has an attractive dark brown to black heartwood, often striped, durable and dense (Keay *et al*., 1989). Its seeds germinate readily with high viability.

1.18.3. *Bauhinia monandra* (Kurz)

Bauhinia monandra is a species of leguminous tree, of the sub-family Caesalpinoideae in the Fabaceae family. It is commonly known as the Orchid Tree or Napoleon's Plume. It grows naturally in Madagascar, but has naturalized in many tropical countries, including Nigeria. Nyanayo (2006) reported that *B. monandra* as

the species occurring in the Niger Delta and rainforest region which is contiguous with the Niger Delta region. B. *monandra* is an ornamental tree which is often planted along roads and may be easily recognized by its broad leaves. It has flowers, which have pink or white petals with one large anther, and a sharply elongated pod which is pointed and very persistent. The pods split open explosively (Keay *et al*., 1989).

1.18.4. *Delonix regia* (Hook.) Raf.

Delonix regia is a species of flowering plant in the family Fabaceae, sub-family Caesalpinioideae. This ornamental tree is commonly referred to as 'Flamboyant' or Flame of the Forest in Nigeria (Aigbokhan, 2014). It is noted for its fern-like leaves and flamboyant display of flowers. It is a fast growing tree species, usually with low and widely spreading branches producing a broad flat canopy (Keay *et al*., 1989). This tree is a legume, and thus has N-fixing and soil-improving properties. *Delonix regia* is commonly propagated by seeds. Seeds are collected, soaked in warm water for at least 24 hours, and planted in warm, moist soil in a semi-shaded, sheltered position. It requires a tropical or sub-tropical climate, but can tolerate drought and saline conditions. It prefers an open, free-draining sandy or loamy soil enriched with organic matter. *Delonix regia* is endemic to the western forests of Madagascar, it is very widely grown in the Caribbean, Africa and Northern Australia, but has been introduced into tropical and sub-tropical regions worldwide and occurs abundantly in the Niger Delta region of Nigeria (Nyananyo, 2006).

1.18.5. *Peltophorum pterocarpum* (DC.) K. Heyne

Peltophorum pterocarpum, commonly known as Yellow Flamboyant or Yellow Flame Tree, is an ornamental leguminous tree species of the sub-family Caesalpiniaceaea and family Fabaceae. It is a deciduous fast growing tree which can attain a height of 15-25 m (rarely ≤50 m) tall, with a trunk diameter of ≤1 m (Keay *et al*., 1989; Nyananyo, 2006). The tree is widely grown in tropical regions as an ornamental, particularly in India and Nigeria. It is one of the legumes that grows in the wild forests of the Niger Delta (Osam *et al*., 2008). It has a wide variety of uses, including cabinet-making and the foliage is used for fodder. Osam *et al*., (2008) reported its efficacy in restoration of crude oil-polluted soil.

1.18.6. *Tetrapleura tetraptera* (Schum & Thonn.) Taub

Tetrapleura tetraptera is a leguminous species of the sub-family Mimosoideae and family Fabaceae and it is endemic to tropical Africa and grows well in the secondary forests ((Keay *et al*., 1989; Omokhua and Ukoimah, 2008). It is commonly known as Aridan or Gum Tree and is native to southern Nigeria (Aigbokhan, 2014). It is a deciduous forest tree occurring on the fringe of the rain forest of the Niger Delta. It has compound leaves and attains a height of 20-25 m and girth of ≤1.5 m (Nyananyo, 2006). It improves soil conditions and is a good source of hardwood. It has medicinal and economic values (Omokhua and Ukoimah, 2008).

CHAPTER TWO

Materials and Methods

This chapter focuses on the general methods used in the plant experiments, soil analysis and kaolinite oil-sorption potential and re-usability studies. Experimental procedures for the three experiments reported in Chapters 3-5 are also discussed in this chapter. The schematic representation of the experimental stages is shown in **Figure 2.1.**

2.1. Study sites

Investigations were carried out in the Greenhouse and Postgraduate Laboratories of the Department of Plant Science and Microbiology of Ekiti State University, Ado-Ekiti, Nigeria. Central Research Laboratory of the University of Lagos, Akoka, Nigeria and Faculty of Science and Engineering, University of Wolverhampton, UK.

2.2. Experimental samples

(a). **Soil**: The sandy-loam topsoil used in the study was collected from a 4-year old fallow plot in the Research and Experimental Farm of Ekiti State University, Ado-Ekiti, Nigeria (7º40′N; 5º15′E) at 0-10 cm depth, according to Song *et al*., (1990).

(b). **Crude oil**: Bonny light type of crude oil (**Plate 2.1**) was collected from Agip Petroleum Company, Omoku Flow Station, Omoku, Rivers State, Nigeria. The crude oil was used for the contamination of soil from the location described above, to simulate oil spill onto soil.

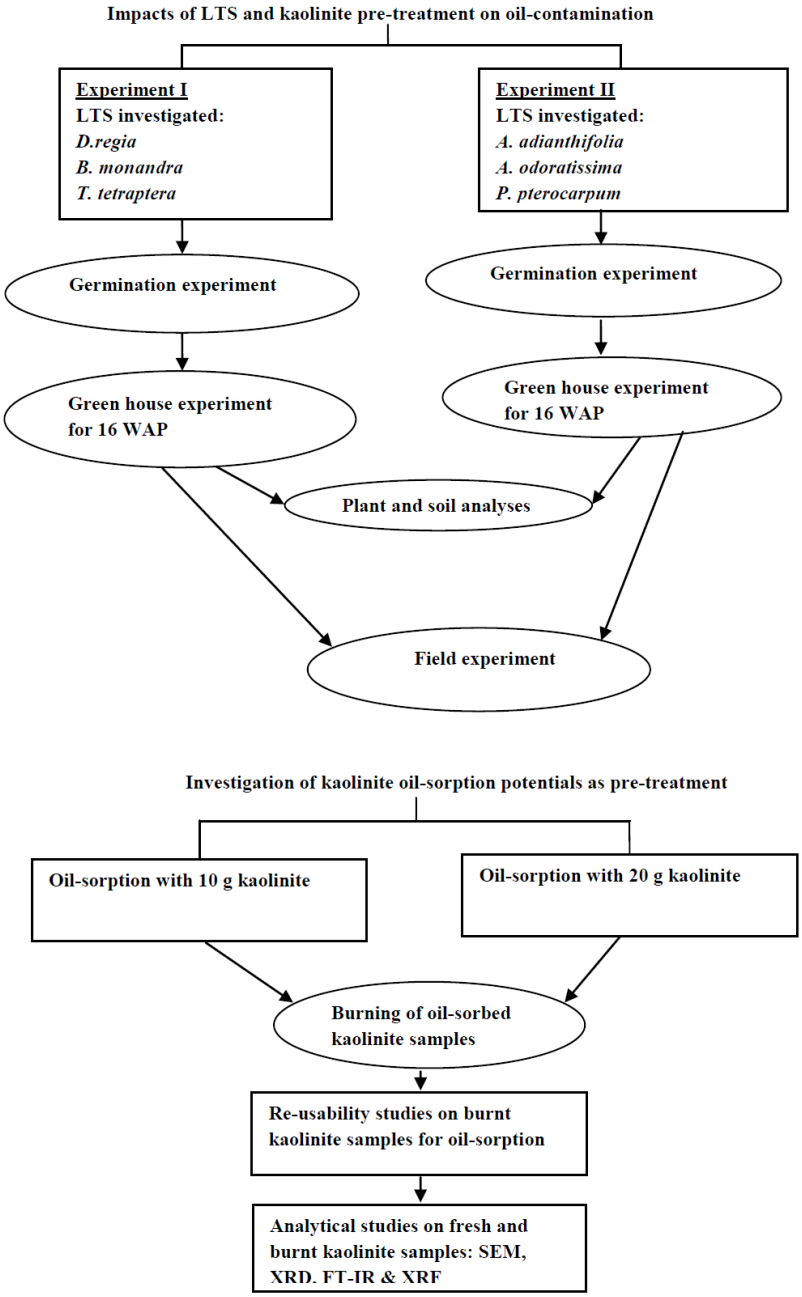


Figure 2.1: Schematic representation of experimental stages on the investigation of impacts of LTS and kaolinite treatment on oil-contamination.

Plate 2.1: A typical Nigerian light crude oil.

(c). **Selected Leguminous Tree Species (LTS) seeds investigated:**

(i) *Albizia adianthifolia* seeds (**Plate 2.2**) were obtained from the forest vegetation along Owan/Benin Road in Edo State (6º30′N; 6º00′E).

Plate 2.2: *Albizia adianthifolia* seeds.

(ii). *Albizia odoratissima* seeds (**Plate 2.3**) were obtained from forest vegetation at Arugbo, Ondo State (7º10′N; 5º05′E).

Plate 2.3: *Albizia odoratissima* seeds.

(iii). *Bauhinia monandra* seeds (**Plate 2.4**) were obtained from forest vegetation at Ifon, Ondo State (7º10′N; 5º05′E).

Plate 2.4: *Bauhinia monandra* seeds.

(iv). *Delonix regia* seeds (**Plate 2.5**) were obtained in a from forest vegetation at Oporoma village, Bayelsa State (4º45′N; 6º05′E).

Plate 2.5: *Delonix regia* seeds.

(v). *Peltophorum pterocarpum* seeds (**Plate 2.6**) were obtained from the Convocation arena, University of Portharcourt, Portharcourt (4º45′N; 6º50′E).

Plate 2.6: *Peltophorum pterocarpu*m seeds.

(vi). *Tetrapleura tetraptera* seeds (**Plate 2.7**) were obtained from vegetation at Uyo, Alkwa-Ibom State (5º00′N; 07º05′E).

Plate 2.7: *Tetrapleura tetraptera* seeds.

2.3. Experimental design

This study involved laboratory, greenhouse and field experiments. Logistically, three plant species were investigated each year. These were *Bauhinia monandra*, *Delonix regia* and *Tetrapleura tetraptera* in 2013 and *Albizia adanthifolia*, *Albizia odoratissima* and *Pterophorum pterocarpum* in 2014. Analyses were conducted on randomly-selected soil samples and were used for the determination of the biological and physico-chemical properties of the uncontaminated (control), contaminated and LTS-planted soil samples at 16 weeks after planting.

2.4. Viability test of seeds

Seed viability is an important factor that determines seed germination and plant growth. Plants that can grow well in crude oil contaminated soil have been described as potentially suitable species for the phytoremediation of (Bamidele and Agbogidi,

2006). It therefore, became imperative to determine the viability of the seeds grown in this study. The floating method, according to Anoliefo and Vwioko (1995), was used to determine the viability of LTS in this study. A sample of 500 seeds of each plant species were soaked in a water bath that contained distilled water for 30 minutes. The seeds that floated were discarded and 300 viable seeds were selected out of the seeds that sank.

2.5. Geography of the study site in Nigeria

The study was conducted in the 'Green House and Postgraduate Laboratories of the Department of Plant Science and Microbiology', Ekiti State University, Ado-Ekiti (Nigeria) in 2013 and 2014. Ado-Ekiti, (7º40′N; 5º15′E) is the capital city of Ekiti State has a tropical humid climate with two distinct seasons: a relatively cool wet season, which lasts from March-October (with a short dry season in July and August) and hot dry season between November-February. The area falls within the forest zone where the rich tropical forests thrive. Ado-Ekiti has abundant rainfall (mean 1367 mm annually) with a mean daily temperature of 27ºC (Ademiluyi and Omotoso, 2008) and the town is >400 m above sea level. Kayode and Faluyi (1994) described the site soil as overlying metamorphic rocks of basement complex (utisol) which shows greater variation in grain size and mineral composition.

2.6. Germination experiment of the selected LTS

Five medium sized (2000 cm^3) plastic plant pots were filled with sandy loam topsoil from the location described in **Section 2.2**. The plant pots, with a uniform weight of 4000 g were arranged in the greenhouse. The soil was artificially contaminated with varying amounts (0, 25, 50, 75 and 100 ml in 4000 g soil) of crude oil and

thoroughly mixed in a large basin. The varying amount of light crude oil in soil represents the treatments (uncontaminated, low, moderate, high and very high contamination) and their concentrations were 0.0, 0.63, 1.25, 1.88 and 2.5 %v/w, respectively (**Appendix 2.1**). These contamination levels were uncontaminated (0.0 ml), low (25 ml), moderate (50 ml), high (75 ml) and very high (100 ml) levels of contamination as related to 50 mg kg^{-1} compliance limits (DPR, 1991). Samples of 200 g of contaminated soil were removed from each treatment using a weighing balance (DTA Series Electronic Balance FED-3000) and each was placed into a 1000 ml measuring cylinder (Technico, UK) containing distilled water and made up to the 1000 ml mark. Soil samples were left to soak for 72 hours. Aqueous extracts were filtered using Whatman No. 1 filter paper and the filtrates were collected in 500 ml conical flasks (Pyrex, UK). The plant species: *B. monandra, D. regia* and *T. tetraptera* were investigated in 2013, while *A. adanthifolia*, *A. odoratissima* and *P. pterocarpum* were studied in 2014. In each year, 75 sterile Petri dishes were each double layered with Whatman No 1 filter paper (Whatman International Ltd, Maidstone, UK) and divided into three groups; a group for each plant species (**Plate 2.8**). In each group, the treatments were replicated five times. Ten seeds of each plant species A. *adanthifolia, A. odoratissima, B. monandra, D. regia, P. pterocarpum* and *T. tetraptera;* all of the Fabaceae family) were sown in each Petri dish and moistened daily at 0700 for 10 days to determine seed germination. Germination counts were made daily and recorded for 10 days after sowing. Germination in each treatment and its control was calculated and mean values compared. Percentage germination was calculated, modifying the formula adopted by Kayode and Oyedeji (2012):

(*Gt %) = Number of seedling that emerged/dish ×100
Total number of seed sown. 2(1)

*Germination test Percentage.

The Co-efficient of Velocity (COV) of germination in each treatment was determined according to Chaco and Singh (1966) and Kayode (2000) as:

$$\text{Coefficient of Velocity (COV)} = \frac{A_1 + A_2 + \ldots\ldots + A_{10}}{A_1T_1 + A_2T_2 + \ldots\ldots + A_{10}T_{10}} \times 100 \qquad 2(2)$$

Where A is the number of seeds germinating and T is the number of days taken to germinate.

The data obtained were compared to those of controls using relevant statistical analyses at $P<0.05$.

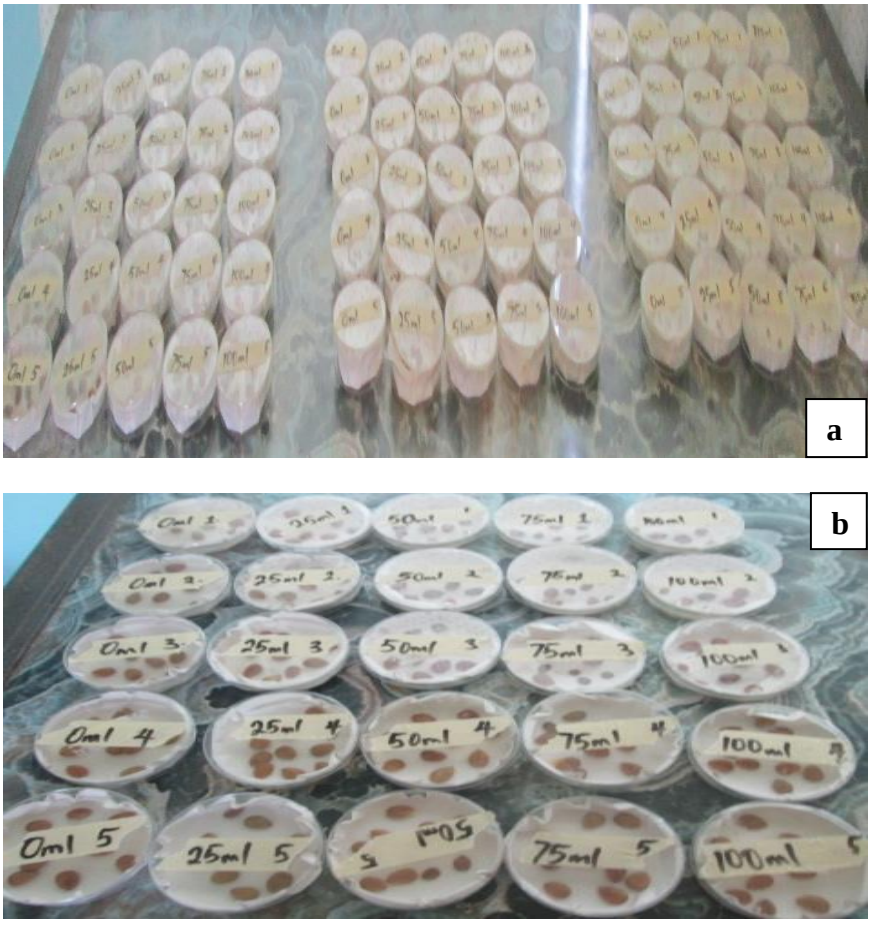


Plate 2.8: Layout of germination experiment of the selected LTS: Layout for three LTS (b) Layout for single LTS.

2.7. Greenhouse experiment: Establishment of selected tree species in crude oil-contaminated soil

Fifty (50) medium sized (2000 cm^3) plastic plant pots were filled with topsoil. Each planting pot with a uniform weight of 4000 g was arranged in the greenhouse (**Plate 2.9**). They were divided into five sub-groups. Each sub-group consisted of 10 pots, arranged in a row. The groups were contaminated with different concentrations (0.0, 0.63, 1.25, 1.88 and 2.5 %v/w) of light crude oil. All the treatments and control pots were watered with 500 ml distilled water for two weeks at an interval of 72 hours at 0700 and one viable seed was sown in each of the pots in the treatments and its control two weeks after oil contamination.

Plate 2.9: Layout of greenhouse experiment for three LTS.

Evaluation of the tree species agronomic parameters (plant height, girth and leaf number) was conducted every two weeks for 16 weeks. The seedling height (**Plate 2.10a**) was taken using a meter rule between the soil level and the last node towards the upper (aerial) part of the shoot. Seedling girth (**Plate 2.10b**) was determined at

first node above the soil level with the aid of a Vernier caliper. The number of leaves produced by the seedlings growing in the control and oil-contaminated soil were physically counted. These paramters were similarly used by Vaitkutė *et al*. (2010) to determine tree growth.

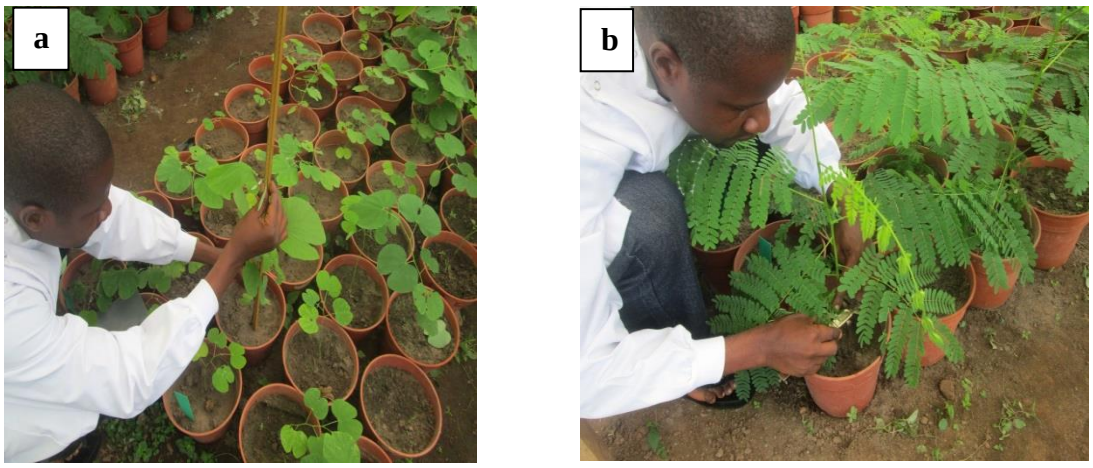


Plate 2.10: Determination of growth parameters of selected LTS: (a) plant height (b) plant girth.

Plate 2.11: Nodulation in the selected LTS grown in crude oil-contaminated soil.

The seedling in each planting pot of each treatment was carefully harvested and roots carefully washed in distilled water to remove soil particles at 16 weeks after planting (16 WAP). Nodulation in the tree species (**Plate 2.11**) was determined by physically

counting the number of nodules produced in seedlings and thereafter root and shoot biomass were determined (**Plate 2.12**). Seedlings were separated into shoot (part above soil level) and root (part below soil level). Then the separated shoots and roots from each treatment were air-dried at room temperature for one week and enveloped separately and their dry weights were determined and recorded using electronic scale Model Scout Pro SPU2001series.

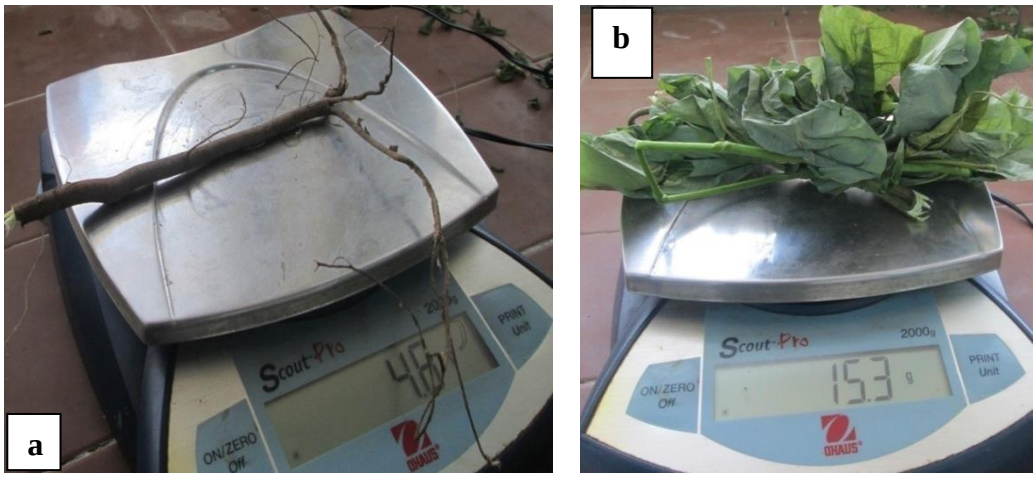


Plate 2.12: Determination of root and shoot biomass (a) root (b) shoot of the LTS.

2.8. Soil analyses

The uncontaminated soil used in the experiment was analysed for its physico-chemical properties. Contaminated soil was also analysed after contamination at an interval of 4 week for 16 WAP. Soil biological properties were also investigated, especially soil bacteria and fungi in Experiments I and II.

2.8.1. Soil sample preparation

Soil samples were analysed for pH, Soil organic carbon (SOC), available sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), nitrogen (N) and phosphorus (P). Samples were prepared using the following method: 72 hours air-dried soil

samples (at room temperature) were passed through a 2.0 mm sieve. Extraneous materials and soil particles remaining in the sieve were also broken down with a pestle and mortar, until all aggregates were disrupted and only small stones (2.0 mm) were left behind on the sieve surface.

2.8.2. Analysis of soil physical properties

2.8.2.1. Determination of bulk density

The presence of oil in agricultural soil can have profound effect on the soil physical and chemical properties. It can cause an undue compaction of soil particles and therefore decrease porosity and aeration. The bulk density of soil samples was determined according to Ibitoye (2006). Cylindrical bulk density metallic soil sampler tins of 5.0 x 5.1 cm dimensions, opened at both ends and with a volume of 100 cm^3 were used to determine the bulk density of the field soil samples. The weight of the empty metallic cylindrical soil sampler was determined and recorded as W_1 in the laboratory. The samplers were pressed vertically into the soil surface at three different locations, from the soil surface and were carefully removed with its soil content with the aid of a metal trowel. After evacuation of the soil samplers, soils extended from both ends of the tins were trimmed with a sharp knife and metal caps placed on both ends. The tins were then placed in zip lock plastic bags to retain moisture. The weight of the sampler plus soil content was also determined and recorded as W_2. Samples were then taken to the laboratory and weighed wet before the sampler plus its soil content was oven dried at 105ºC for 72 hours. The soil was allowed to cool and weight was determined and recorded as W_3. The volume (V) of the sampler was determined using the relationship $\pi r^2 h$ as 100 cm^3. Bulk density of soil sample was thus determined using the relationship below:

$$\text{Bulk Density (BD)} = \frac{\text{Weight of oven-dried soil (g)}}{\text{Volume of oven-dried soil (cm}^3\text{)}} \qquad 2(3)$$

$$\text{i. e.} \quad \frac{W_3 - W_1\ (\text{g/cm}^3)}{V}$$

2.8.2.2. Determination of soil moisture content (MC)

Moisture is a critical environmental variable. It affects soil respiration, and, as such, the environment must contain sufficient water for maximum plant growth and microbiological action. Maximum plant growth can be achieved in soils under adequate water conditions. Osuji *et al*., (2006) observed variations in the moisture content of crude oil-contaminated and non-crude oil-contaminated soils collected from the same area. The fact that soil moisture content plays a unique role in plant and microbial growth and development, it was necessary to determine the moisture content of the soil used in this experiment. Percentage moisture content was estimated according to Osuji and Onojake (2004). A known amount of the dry site soil sample was weighed into the crucible and heated in an oven at 105ºC overnight. The sample was then allowed to cool in desiccators containing P_2O_5 for 1 hour and then re-weighed. The percentage moisture content (% MC) was calculated by calculating the loss in drying as a fraction of the initial mass of sample and multiplied by 100.

$$\%\ \text{MC} = \frac{[\text{Weight of wet soil (g) - Weight of dried soil (g)}] \times 100\%}{\text{Weight of wet soil (g)}} \qquad 2(4)$$

2.8.2.3. Determination of Soil Water Capillarity

Soil water capillarity (i.e. the rate at which water rises above the water-table in the soil) was determined in soils using glass capillarity tubes open at both ends (**Plate**

2.13) (Kayode *et al*., 2009b). 500 ml capillarity tube corked with cotton wool at one end was filled with the 200 g of the uncontaminated soil sample. The tubes were inserted into a 1000 ml beaker (Pyrex, UK) and the tubes clamped on a retort stand. A stopwatch was set in operation and the total volume of water absorbed by the soil in each tube, along its vertical length, was determined over a 60 minute period. The volume of water absorbed in a vertical upward direction (uptake) was determined on the graduation along the tubes.

Plate 2.13: Experimental determination of soil water capillarity.

2.8.2.4. Determination of soil porosity

Soil interstices influence the passage of water, nutrients and air and therefore influence plant growth and aeration. Soil porosity (**Plate 2.14**) was determined according to Akinsanmi (1975), as adopted by Kayode *et al*., (2009b), by measuring

out 100 g of uncontaminated soil sample and oven-drying at 80ºC for 48 hours. A sheet of Whatman No 1 filter paper (Whatman International Ltd, Maidstone, UK) was folded into a cone shape, placed in a glass funnel (Pyrex, UK) and filled with the oven-dried soil sample. Then, 200 ml of distilled water was added and the water was allowed to drain for 24 hours. The volume of drained water over the stated period was determined and recorded using the relationship below:

Soil porosity (SP) = Initial water volume – Water drained from soil (ml) 2(5)

The soil porosity was also cross-checked using the relationship adopted by Ewetola (2013):

Total porosity (%) = 1 – (BD/PS) x 100 2(6)

Where BD is bulk density (g/cm^3) and PS is the particle density (g/cm^3).

Most soils, including tropical soils have a particle density of 2.65 (Landon, 1991). This value is most suitable for soil used for agricultural activities. It should be noted that soils with large ferric oxide content can have a slightly dissimilar particle density. However, the difference is extremely small and therefore 2.65 give a sufficiently accurate estimate of particle density in most soils.

Plate 2.14: Experimental determination of soil porosity.

2.8.3. Analysis of soil chemical properties

2.8.3.1. Determination of soil pH

The pH of a solution, or soil-water suspension, determines its degree of acidity or alkalinity. pH is the negative logarithm to base-ten of hydrogen ion concentration [H^+] in a given solution. Soil pH usually falls within the ranges of 4.0-8.5 or in extreme cases 2.0-10.5. It was necessary to determine the pH of the uncontaminated and crude oil-treated soil samples to determine soil acidity level, as most plants grow optimally within soil-water pH values 5.5-7.0 (Odokuma and Dickson, 2003; Osuji and Adesiyan, 2005). Soil pH can vary widely and controls plant nutrient availability and microbial reactions in the soil. It is therefore a fundamental factor that influences the availability of plant nutrients. Soil pH affects plant species differently. The pH of soil influences the population and types of soil organisms that

change plant residues into valuable Soil Organic Matter (SOM), thereby influencing soil aggregate stability and air and water movement. Soil pH is important in agricultural practise since different crops thrive at varying pH levels. Soil pH is spatially highly variable (Odokuma and Dickson, 2003; Osuji *et al* 2005; Osuji and Adesiyan, 2005). It was, therefore, very important to determine soil pH in this study.

Soil pH was determined using the method of Klute (1965), as reported by Ibitoye (2006). Approximately 10 g of 2.0 mm air-dried soil samples were weighed into a 100 ml beaker and 20 ml of distilled water was added (ratio 1:2 soil to water) and the suspension was stirred thoroughly with a glass rod to form an homogenous slurry and left at room temperature for 20 minutes. Soil pH was determined using a pre-calibrated Jenway 3520 electrode pH meter. Then the glass electrode of the pH meter was well inserted into the partly settled suspension in the beaker and after equilibrium, the pH value of each treatment soil was observed and recorded. The electrode was rinsed with distilled water and wiped dry with clean tissue paper after each reading.

2.8.3.2. Determination of Soil Organic Carbon

The source of SOM is related to the vast quantities of plant remains and forest litter that decompose above the soil surface, subterranean plants and above-ground tissues that are bio-mechanically incorporated into the soil. Other sources of SOM are animal tissues and excretory products as well as flora and fauna carbon. All these contain ~58% organic carbon (Osuji and Adesiyan, 2005). Total organic carbon (TOC) and total organic matter (TOM) contents (% TOC and % TOM) are co-indices of soil fertility (Osuji and Adesiyan, 2005). TOC has a major influence on

both the chemical and biological processes in soils and sediments. The amount of organic carbon has a direct role in determining the redox potential in sediment, thus regulating the behaviour of other chemical species, such as metals. TOC content is proportional to TOM, which has an affinity for trace metals and organic contaminants. Soil TOC and TOM contents also vary spatially (Osuji and Adesiyan, 2005).

The organic carbon content of each soil treatment was determined by chromic acid oxidation method according to Walkey and Black (1934) as reported by Ibitoye (2006). For each soil sample, 10 g air-dried < 2.0 mm soil was ground, 1 g was weighed and poured into a 250 ml conical flask (Pyrex, UK). Approximately 10 ml of potassium heptaoxodichromate (IV) solution ($K_2Cr_2O_7$) was pipetted into the flask and gently swirled to disperse the soil sample subsequently. 20 ml of concentrated textraoxosulphate (VI) acid was rapidly added. The flask was swirled gently until soil and reagents mixed, then swirled more vigorously for 10 minutes and the mixture was allowed to stand for 30 minutes on a sheet of asbestos, after which 100 ml of distilled water was added into the flask. Four drops of Ferroin indicator are added. Then the mixture was titrated with 0.5 M iron (II) sulphate solution, which was added drop-by-drop until the colour of the mixture changed to light green then to dark green and finally to the brownish red-end point. A blank titration was performed in the same way (without soil) to standardize the $K_2Cr_2O_7$. Then the SOC content in each treatment soil was determined. The values obtained for SOC was thus used to determine the SOM content of the soil samples, using the 0.58 constant.

2.8.3.3. Determination of Organic Matter

Soil Organic Matter (SOM) is an important factor that determines plant productivity through influencing soil structure. SOM was calculated by multiplying the organic carbon value obtained in (**Section 2.8.3.2**) above with a correction factor. The calculation is as follows:

$$\% \text{ Soil organic matter} = \frac{MeK_2Cr_2O_7 \times MeFeS0_4 \times 0.03 \times 100 \times F}{\text{Weight of air-dried soil (g)}} \qquad 2(7)$$

Where, F = correction factor = 1.33

M = Mill equivalent (Volume used x concentration of solution)

% Soil organic matter = Soil organic carbon x 1.729.

2.8.3.4. Determination of total soil Nitrogen

The most preferred analytical method for the determination of total nitrogen (N) is the Kjeldahl method (Anderson and Ingram 1996; Omotoso and Shittu, 2007). The method was developed by a Danish chemist, Johan Kjeldahl in 1883. LTS have been reported to increase soil N (Ogunnika and Kayode, 2005). The adverse effect of oil-contamination on soil can therefore be corrected by growing LTS on such soil to ameliorate N deficiency.

Principle

The principle governing the Kjeldahl method can be summarized in three steps:

(a) The soil sample is first digested in strong sulphuric acid in the presence of a catalyst, the catalyst aids the conversion process of amine N to ammonium ions;

(b) Ammonium ions are converted to ammonia gas, heat is then applied followed by a distillation process. The ammonia gas is directed into a trapping solution and it is dissolved and converted to ammonium hydroxide ions in such solution;

(c) The amount of trapped ammonia is determined by titration with a standard solution and total N is calculated.

Method

For each treatment, a 5 g soil sample was weighed into 500 ml digestion flask and two Kjeldahl catalyst tablets were added. Copper sulphate is the most commonly used catalyst and it was used in this experiment. Subsequently, 20 ml tetraoxosulpate (VI) acid was added and the mixture was heated on a Bunsen burner flame and changed into a grey/white colour, which indicated complete digestion of the soil. The soil was then left to cool at room temperature for 30 minutes. Organic N was converted to ammonium-N by the addition of sulphuric acid and the catalyst. The acid digest produced was distilled for 4 minutes in the presence of 40% NaOH. Ammonium ions NH_4^+ were converted to ammonia during this phase and it was then released from the solution by steam distillation and condensed as ammonium hydroxide (NH_4OH) and the distillate is then trapped. 5 ml of 2% boric acid was then added. The distillate was titrated with 0.01/0.1 M HCl until the colour changed from light green to pink, and the titre value was noted for the determination of total N present in the soil samples.

2.8.3.5. Determination of exchangeable cations (Na, K, Ca, Mg) in soil samples

Nutrients such as Na, K, Ca and Mg present in the soil solution are available for plant growth and can be taken up by the roots. Crude oil contamination has been reported to have a deleterious effect on soil exchangeable properties and often time decreases the concentration of soil nutrients (Ogboghodo *et al*., 2004; Tanee and Akonye, 2009; Njoku *et al*., 2009; Osam *et al*., 2011b). However, application of

remedial measures, such as phytoremediation, could restore contaminated soil to a healthy condition over a period of time. Hence, it was necessary to determine the amount of these nutrients present in soil samples. The oil-contaminated soil samples studied were first digested before the determination of exchangeable cations and the procedure followed is stated below.

2.8.3.5.1. Procedures of soil digestion

For each treatment, a 2 g of air-dried < 2.0 mm soil were weighed into a 250 ml conical flask (Pyrex, UK) and 10 ml of aqua regia (HNO_3/HCL in ratio 1:3) was added. The mixture was gently heated on a hot plate at 200ºC and heating continued until the brown fumes turn to white in a fume cupboard (**Plate 2.15**). The conical flask was allowed to cool to room temperature. The mixture was rinsed with 20 ml deionised water and filtered with Whatman No. 1 filter paper into a standard 25 ml volumetric flask and made up to mark. Subsequently, 20 ml of the filtrate was stored in a universal sample bottle for Atomic Absorption Spectrophotometry (AAS) analysis.

Plate 2.15 Soil digestion: (a) Digestion of soil samples (b) Digested soil samples prepared for AAS analysis.

Preparation of stock standards for Sodium (Na)

4.6 g of Na_2CO_3 salt was weighed into a 250 ml Pyrex beaker and dissolved in 100 ml distilled water. The solution was rinsed into a 1000 ml volumetric flask and made up to mark with distilled water. This is expressed as:

$$\frac{\text{Molar mass of } Na_2CO_3}{\text{Molar mass of Na}} = \text{mass of 1 mole of Na in } Na_2CO_3 \qquad 2(8)$$

Therefore: $\frac{106}{23}$ = 4.6 g is equal to 1 mole of Na in Na_2CO_3.

Preparation of stock standards for Potassium (K)

3.5 g of K_2CO_3 salt was weighed into a 250 ml Pyrex beaker and dissolved in 100 ml of distilled water. The solution was rinsed into a 1000 ml volumetric flask and made up to mark with distilled water. This is expressed as:

$$\frac{\text{Molar mass of } K_2CO_3}{\text{Molar mass of K}} = \text{mass of 1 mole of K in } K_2CO_3 \qquad 2(9)$$

Therefore: $\frac{138}{39}$ = 3.5 g is equal to 1 mole of K in K_2CO_3.

Preparation of stock standards for Calcium (Ca)

The stock standard was prepared by weighing 2.5 g of $CaCO_3$ salt and dissolving in 1000 ml of 5% HNO_3. The salt was initially dissolved in a Pyrex beaker after which it was rinsed into a 1000 ml volumetric flask with 5% HNO3 and made up to mark. This is expressed as:

$$\frac{\text{Molar mass of } CaCO_3}{\text{Molar mass of Ca}} = \text{mass of 1 mole of Ca in } CaCO_3 \qquad 2(10)$$

Therefore: $\frac{100}{40}$ = 2.5 g is equal to 1 mole of Ca in $CaCO_3$.

Preparation of stock standards for Magnesium (Mg)

The stock standard was prepared by weighing 5 g of $MgSO_4$ salt and dissolving in 50 ml of 5% HCl. The salt was initially dissolved in a beaker after which it was rinsed into a 1000 ml volumetric flask with 5% HCl and made up to mark. This is expressed as:

$$\frac{\text{Molar mass of } MgSO_4}{\text{Molar mass of Mg}} = \text{mass of 1 mole of Mg in } MgSO_4 \qquad 2(11)$$

Therefore $\frac{120}{24}$ = 5 g is equal to 1 mole of Mg in $MgSO_4$.

Serial dilution of stock standards

The stock standards prepared were serially diluted to concentrations of 5, 10, 15, 20 and 25 ppm. These different standard calibration levels were used to generate a suitable curve, which was used to calibrate the instrument using the serial dilution formula:

$$C_1V_1 = C_2V_2$$

Where, C_1 is the initial concentration

C_2 is the final concentration

V_1 is the initial volume

V_2 is the final volume

After the serial dilution of stock standards the different calibrants' were fed into the AAS as standard samples. These were used by the AAS to generate a suitable calibration curve prior to sample analysis.

2.8.3.5.2. Procedures for determining exchangeable ions (K, Na, Ca, Mg)

The concentration of the exchangeable cations ions: K, Na, Ca, Mg in the treated soil samples were determined by Atomic Absorption Spectrophotometry (AAS) (**Plate 2.16**) from acid digests, as reported by Osam *et al*., (2008) and Ademiluyi and Omotoso (2008). The estimation of the level of the exchangeable metals in the crude oil-contaminated soil was determined using AAS (PerkinElmer AAnalyst, 200) and equipped with K, Na, Ca and Mg Lumina hollow cathode lamps. Digested samples were aspirated in the Nebulizer chamber. In the chamber, the sample air and fuel mixed together and formed an aerosol. About 10% of the aerosol went into the flame and 90% went out as waste. The flame vapourized, burned and atomized the samples from the ground state to the excited state. The monochromator selected the wavelength in agreement with the atom that comes in based on the light source (i.e. PerkinElmer Lumina hollow cathode lamp) of the various exchangeable cations. The detector detected the atom and transfers the reading to the read out (desktop computer) attached to the AAS instrument. K, Na, Ca and Mg were determined at wavelengths 766.5, 589.0, 422.7 and 285.2 nm, respectively.

Plate 2.16: Determination of exchangeable cations using Atomic Absorption Spectrophotometer.

2.8.3.6. Determination of soil phosphorus

Available P in soil samples were extracted by the Brays method and determined colorimetrically (Bray and Kurtz, 1945). Approximately 1 g of soil sample was weighed into a 15 ml centrifuge tube and 7 ml of extracting solution (NH_4 + HCl + distilled H_2O) were added. The mixture was shaken for 1 minute and centrifuged at 2,000 rpm for 15 minutes. Subsequently, 2 ml of the clear supernatant was pipetted into a 20 ml test tube and was followed by 5 ml of distilled water. Further, 2 ml of ammonium molybdate solution [$(NH_4).MO_7O_2.4H_2O$] was added. The resultant solution was mixed thoroughly and 1 ml of dilute stannous chloride ($SnCl_2.2H_2O$) solution was added and mixed thoroughly. The mixture was left for 20 minutes and then passed through the spectrophotometer at 660 nm wavelengths to measure % transmittance using distilled water as blank. The absorbance of the soil sample was taken thereafter at 660 nm using series of working standard dilutions as a reference solution. A standard curve from which the optical density (o.d) of the standard solution against the concentration was prepared. Phosphorus concentration in the sample was extrapolated from a standard curve and calculated using the relationship:

$$P\ (\%) = \frac{C\ (mg) \times \text{vol. of solution (ml)}}{10 \times \text{aliquot (ml)} \times \text{sample weight (g)}} \times 100 \qquad 2(12)$$

Where:

C = P obtained from the graph
Vol. of solution = 7 ml
Aliquot = 2 ml
Sample weight = 1 g.

2.8.3.7. Determination of Total Petroleum Hydrocarbon (TPH) contents of soil samples using gas chromatography

Total hydrocarbon content (THC) of soils gives an empirical insight into the level of hydrocarbon pollution on site. Hydrocarbons are known to be the major components of crude oil and petroleum products (Britton, 1984), and their presence in the environment above regulatory consent limits indicates pollution. A compliance baseline limit of 50 ppm is set for petroleum industries in Nigeria (DPR, 1991). Usually, higher amounts of petroleum hydrocarbons on the site create anoxic conditions in the top surface and sub-surface diffusion and increase the presence of anaerobic organisms, which depletes available oxygen and increases stress to organisms (both top surface and subterranean biota). Some of the soil biota may eventually die of suffocation (Osuji 2001; Osuji *et al*., 2005). To determine the concentration of petroleum hydrocarbons present in the soil samples, it was imperative to first extract the hydrocarbons present in the soil.

2.8.3.7.1. Extraction procedure of total petroleum hydrocarbons (TPH) contents of the soils

The TPH was extracted in dichloromethane and reconstituted in hexane using a separating funnel. For each treatment, 2 g of soil was weighed into the funnel and 20 ml of dichloromethane added. The mixture was shaken vigorously and left on a retort stand for 1 hour. The extract was collected by filtering into a quartz beaker and transferred into a borosilicate glass bottle, the process were repeated twice. The aliquots collected were concentrated on a steam bath to 5 ml and were then exposed to atmosphere to almost dryness and later re-constituted with hexane to 2 ml. This was purified by passing through a pasture pipette packed with anhydrous sodium

sulphate on a membrane (to absorb the remaining water that may be present) and extracts were collected in vials in readiness for gas chromatography analysis.

2.8.3.7.2. Extraction procedure of total petroleum hydrocarbons (TPH) contents of plants

The seedlings grown in the oil-contaminated soil treatments were carefully harvested and roots carefully washed in distilled water to remove soil particles at 16 weeks after planting (16WAP) and were air-dried at room temperature for 1 week. Seedlings were chopped and homogenized with a blender, adopting the method of Atagana, (2011) and extracted using dichloromethane and reconstituted in hexane, similarly to procedures followed for soil extraction.

2.8.3.7.3. Preparation of standards for GC-MS analysis

Aliphatic hydrocarbon defining window (C_8-C_{40}, 35 components, 500 µg/ml in Chloroform) and polyaromatic hydrocarbon (PAH Mix, 17 components, 2.0 mg/ml in CH_2Cl_2:Benzene) standards were purchased from Applied Analytical Services, Lagos, Nigeria. The polyaromatic hydrocarbon from 500 µg/mL was prepared by using a serial dilution formula: $C_1V_1 = C_2V_2$ into 5 ml. Using this formula, 100, 50, 25 and 12.5 µg/ml stocks were prepared as: 100 µg/ml = 100 * 5/500 = 1 ml. Then, 1 ml of stock solution was taken in 5 ml volumetric flasks and made up to mark with diluents and the standard was dissolved. Subsequently, 50, 25 and 12.5 µg/ml was prepared from 100 µg/ml using the same serial dilution process. Similarly, the aliphatic hydrocarbon was prepared following the same procedure. Equal volumes of aliphatic and aromatic standards were mixed for 100, 50, 25 and 12.5 µg/ml and

these standards were used to calibrate the gas chromatograph for TPH determination in the soil and plant samples.

2.8.3.7.4. The determination of Total Petroleum Hydrocarbons (TPH)

The determination of TPH contents were carried out using gas chromatography (GC) (**Plate 2.17**) as reported by White *et al.* (2006) and Liao *et al*. (2015). A gas chromatograph mass spectrophotometer (Agilent Technologies Model 7890AGC system; Agilent Technologies 5975C VL MSD and Automatic Injector model Agilent Technologies 7683B Series) was used to determine TPH in soil samples. For each treatment, 1 µL of extract was injected into a Agilent Technologies Model 7890AGC system; Agilent Technologies 5975C VL MSD and Automatic Injector model Agilent Technologies 7683B Series with the following operational conditions: Column model was Agilent Technologies HP5MS with 30 m length, internal diameter of 0.320 mm and the thickness of the column is 0.25 µm. The column was the stationary phase, while helium was the mobile phase. Initial temperature was 40ºC to hold for 5 minutes and flow rate 15º/minute (Ramp 1) to the temperature of 210ºC to hold for 5 minutes and the flow rate 15º/minutes (Ramp 2) to the final temperature of 280ºC to hold for 10 minutes. The final run time is 36 minutes. The temperature of the heater was 250ºC and pressure is 11.654 psi. Septum Purge flow is 3 ml /minute. Average velocity was 7.1988 cm/sec and flow is 3.9411 mL/minute. The GC recorder was interfaced to a computer interpretation and the chromatograms quantified with reference to standards.

Plate 2.17: Gas Chromatographic Mass spectrophotometer used in the determination of Total Petroleum Hydrocarbons (TPH).

2.8.4. Determination of microbial load in soil samples

Micro-organisms, such as bacteria and fungi, are unicellular microscopic organisms and are widely distributed within the environment. The soil is the fundamental natural habitat for micro-organisms (Okaka and Okaka, 2001). Soil micro-organisms can be sensitive biological markers and can be used to assess soil quality and the degradation of contaminants in soil. Thus, microbiological properties can serve as Soil Quality Indicators (SQLs) because after plants, soil micro-organisms are the second most important biological agent of agricultural ecosystems (Dick, 1994). Microbial degradation (biodegradation) is a complete process facilitated by appropriate ambient and seasonal conditions, the presence of the optimum concentration of the right type of nutrients and the composition of the indigenous

microbial community (Leahy and Colwell, 1990). Soils contaminated with oil and its derivatives are rich in microbial communities capable of surviving toxic contamination. Micro-organisms are sensitive to fluctuations/changes in their environment. Whenever their chemical or physical environment is suddenly altered, there is a lag-period during which the microbial community adapts to the new conditions (Leahy and Colwell, 1990; Chikere and Okpokwasili, 2004; Nweke and Okpokwasili, 2004). This lag-period is also called the acclimatization period and enables micro-organisms to acquire necessary metabolism for their survival in the contaminated soil (Head *et al*, 2006; Yakimov *et al*, 2007).

Bacterial communities in soil and sediments are instrumental in the terrestrial ecosystem, where they are responsible for the recycling of nutrients and degradation of pollutants (Head *et al*, 2006) and similar process occur in terrestrial ecosystems. Several studies have revealed that the bacterial community composition in hydrocarbon-contaminated soil and sediments tend to comprise mostly bacteria that are specially adapted to use hydrocarbons as carbon sources (Kassai, *et al*, 2002). Information on the composition of bacterial populations in a polluted site is valuable in order to estimate the self-purification capability of the ecosystem and the feasibility of biological decontamination if engineered bioremediation is considered (Allen *et al*., 2007, Said *et al*., 2008).

The soil sample from the Research and Experimental Farm of Ekiti State University, Ado-Ekiti; was the main focus of this research and emphasis was on bacteria and fungi counts present in soil samples. The microbial counts were performed on nutrient agar using the standard plate count technique (Oluyege *et al*., 2011).

Collection of sample

For each treatment, soil samples were collected at 0-10 cm depth from randomly selected plant pots at 16 WAP and placed into sterile sampling bottles. The sampling bottles were all placed in an ice-packed cooler and transported to the Laboratory of the Department of Microbiology, Ekiti State University, for microbial counts determination.

Sterilization of materials/disinfection of working area

The following materials were used in the microbial counts investigation: refrigerator, incubator, oven, autoclave, conical flasks, spirit lamp, pipette, culture tubes, petri-dishes, hockey stick, inoculating loop, water bath, measuring cylinder, glass rod, beaker, test tube, test tube rack, soil samples, sterile distilled water, ethanol, inoculating needle, Bunsen burner, sterile plastic bowl, masking tape, aluminum foil, non-absorbent cotton, spatula and detergent. Glassware were soaked in distilled water for 30 minutes and properly washed in a solution containing detergent, rinsed thoroughly in clean distilled water and air-dried at room temperature, after which they were wrapped with aluminum foil for sterilization. The sterilization was carried out using the autoclave at 121ºC and 15 psi for 15 minutes. The procedure was used for all autoclave treatments. The working area and work benches were properly swabbed thoroughly before and after each day work, using 70% ethanol and cotton wool, to prevent all forms of contamination to the work and exposure of Researcher and Technologists to potential pathogenic microbes.

2.8.4.1. Preparation of media

The media used were prepared, sterilized and used according to manufacturer's instructions, by a moist heat sterilization method using an autoclave. The media used

included: nutrient agar (NA), potato dextrose agar (PDA) and buffered peptone water (BPW).

Buffered peptone water (BPW)

Approximately 20 g of the dehydrated powder of buffered peptone water (Oxiodthermo Fisher, UK) were dissolved in 1000 ml distilled water and mixed thoroughly. It was first boiled to aid even distribution of the content. Subsequently, 5 ml of the mixture was then dispensed into head-cap test tubes each.

Nutrient agar (NA)

Approximately 28 g of the dehydrated powder of nutrient agar was weighed, dissolved in 1000 ml distilled water and mixed thoroughly. It was first boiled in the water bath to aid adequate distribution of the agar and later sterilized in the autoclave. This medium was used to isolate and enumerate the total bacteria counts in the soil samples.

Potato dextrose agar (PDA)

Approximately 28 g of the dehydrated powder of nutrient agar was weighed, dissolved in 1000 ml distilled water and mixed thoroughly. It was first boiled in the water bath to aid adequate distribution of the agar and later sterilized in the autoclave. This medium was used to isolate and enumerate the total fungi and yeast counts in the soil samples. The prepared agar media were allowed to cool and then poured into 120 sterile petri-dishes: 60 petri-dishes for each of NA and PDA.

2.8.4.2. Determination of total microbial count

The standard plate count method (Oluyege *et al.*, 2011) was used for the enumeration of total microbial counts in soil samples. Approximately 1 g of soil from each of the treatments was weighed and suspended in 9 ml of 0.1% sterile

buffered peptone water solutions, forming a ratio 1:9 w/v and shaken vigorously in the digester to enable even distribution of organisms. The above suspension formed a 10^{-1} dilution and serves as the primary dilution. It was then incubated at 37ºC for 2 hours, to aid multiplication of organisms present in samples. Subsequently, 1 ml was then taken from 10^{-1} dilution using pipette into another 9 ml sterile buffered peptone water and became 10^{-2} dilution. In all, 10-fold dilutions were prepared, using 1 ml of each primary dilution following vigorous shaking of the contents in the digester.

Aliquots of 1 ml of the dilution 10^{-2} and 10^{-6} for PDA and NA, respectively, of each soil sample were selected and introduced into molten prepared nutrient media (NA and PDA) contained in the labelled Petri dishes with the aid of a sterile hockey stick and the innoculum was spread evenly to prevent overlap of the pore cells and thereby grow on the media (**Plate 2.18a**). Plates were allowed to set on the bench and inverted (**Plate 2.18b**). Inoculated plates containing (NA) were incubated at 37ºC for 48 hours to isolate bacteria counts, while those containing (PDA) were incubated at 37ºC for 72 hours for the isolation of fungi (**Plate 2.18c**). At the end of the incubation periods, the number of developed colonies on the agar was counted and plates having 30-300 colonies (**Plate 2.18d**) were selected for enumeration.

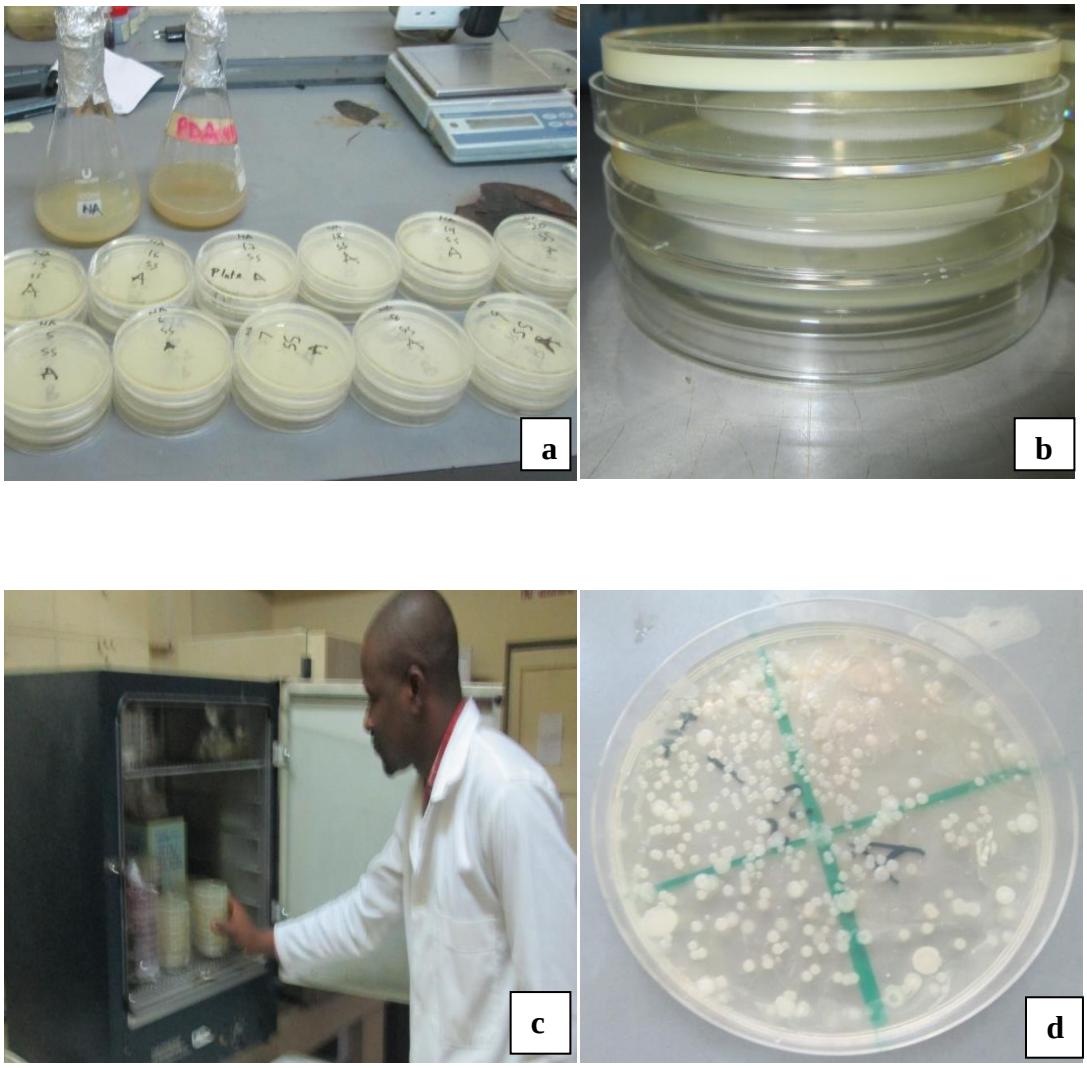


Plate 2.18: Microbial count determination: (a) Microbial inoculums growing on NA and PDA (b) Inverted petri dishes containing prepared cultures of inoculums (c) Incubation of microbial cultures on NA and PDA (d) Culture with grown microbes.

The total microbial counts were obtained by multiplying the number of colonies by the dilution factor and were recorded in Colony Forming Unit per gram (CFU/g) according to Olutiola *et al.* (1991) and Ngari *et al*. (2013) as expressed in equation 2(13). Fresh culture of the developed colonies were sub-cultured onto fresh molten media (NA and PDA) until pure cultures were obtained.

$$\text{Population density (cfu/ml)} = \frac{\text{Number (mean of 3 replicates) of colonies x DF}}{\text{ml of aliquots plated}} \quad 2(13)$$

DF = Dilution Factor.

Sub-culturing and isolation of distinct colonies

Plates having appropriate numbers of colonies were taken for further enumeration. These plates were studied in detail to distinguish several observable distinct colonies, and each of the colonies were sub-cultured onto another plate containing ~15 ml of sterile molten NA (for bacteria) and PDA (for fungi) using the streaking method, under aseptic conditions following proper flaming in a loop. The sub-cultured plates of bacteria were incubated at 37ºC for 48 hours, while that of fungi were incubated at 37ºC for 72 hours. The pure culture of microbial colony, preferably the one that grew separately from other colonies on the sub-cultured plates was selected, transferred and maintained in a Bjoule bottle (for bacteria) and MacCathney bottle (for fungi) containing ~15 ml of sterile molten NA and PDA slants at 4ºC, respectively.

2.8.4.3. Identification of microbial isolates

Identification of isolates is an essential procedure employed to determine whether or not a microbial isolate belongs to any of the already established taxonomic groups and naming them. In this study, standard techniques were used to characterize and identify microbial isolates obtained from the cultures. The bacterial isolates were identified according to Fawole and Oso (2004). Fungal isolates were identified according to the methods of Compbell and Stewart (1980) and Baird *et al.* (2002) using the physiological and morphological features of isolates. Morphological characteristics (colour, shape of colonies, surface elevation, consistency, margin and

Gram stain microscopy) were used for bacteria, while identification of fungi was performed using morphological characteristics and staining.

The bacterial identification in the soil samples was carried out using the following procedure. One drop of sterile water was added to a sterile glass slide. An inoculum was picked with the aid of a sterile inoculating loop and smeared evenly on the slide and passed through a Bunsen flame to pre-fix the smear. The smear was flooded with crystal violet for 1 minute; excess stain was rinsed off using flowing water. Iodine solution was then added for 1 minute and rinsed off again, as stated earlier. The smear was de-colourized using 70% ethanol and rinsed with water. Excess water from the smear was drained off by putting the slide in between folded Whatman No 1 filter paper (Whatman International Ltd, Maidstone, UK). The slide was observed under oil immersion using both low and high light microscopy. Fungal identification in each of the soil samples was carried out using the following procedure. One drop of methylene blue was added to a grease-free glass slide. A fungal inoculum was picked aseptically with the aid of sterile inoculating needle onto the stain and the glass slide was properly covered with cover slip. The slide was observed under oil immersion using both low and high light microscopy (**Plate 2.19**).

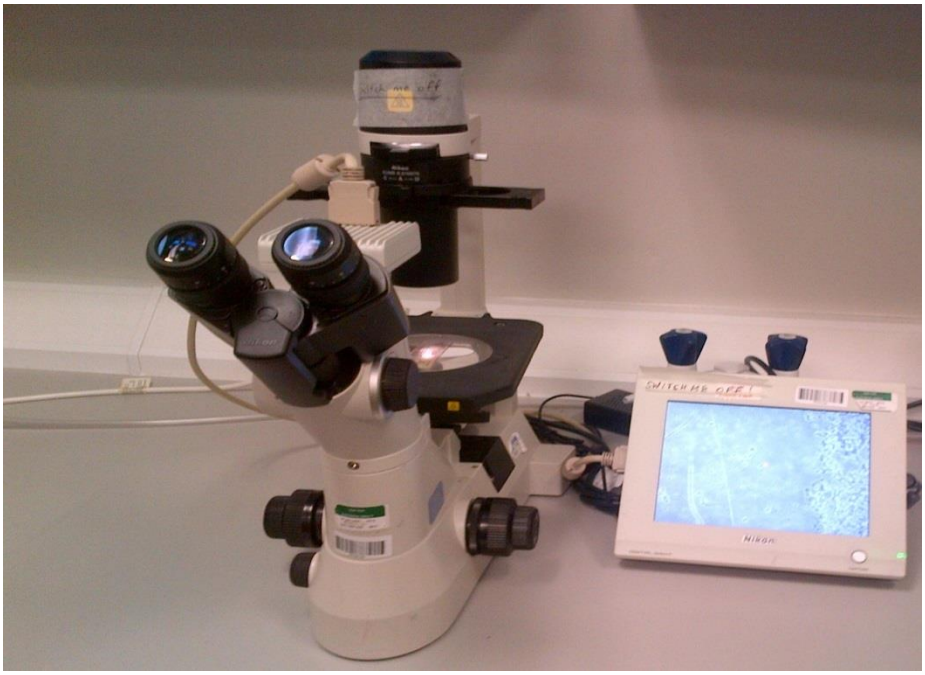


Plate 2.19: Microscopic examination of slides to determine microbial species in crude oil-contaminated soil planted with LTS.

2.9. Field experiment at the Forest of Ayodele

A micro-plot measuring 10 x 12 m located within the Ekiti State University, Ado-Ekiti (7º40′N; 5º15′E) was acquired for the field establishment of the selected LTS. The plot was cleared on 15/09/13 with the aid of a sharp cutlass and dried weeds were packed off the plot 2 weeks after clearing. 15 holes were dug at the depth of 1 m on the micro-plot at an interval of 5 m along the row and 2 m along the column leaving a space of 1 m each sideway of the plot (**Plate 2.20**).

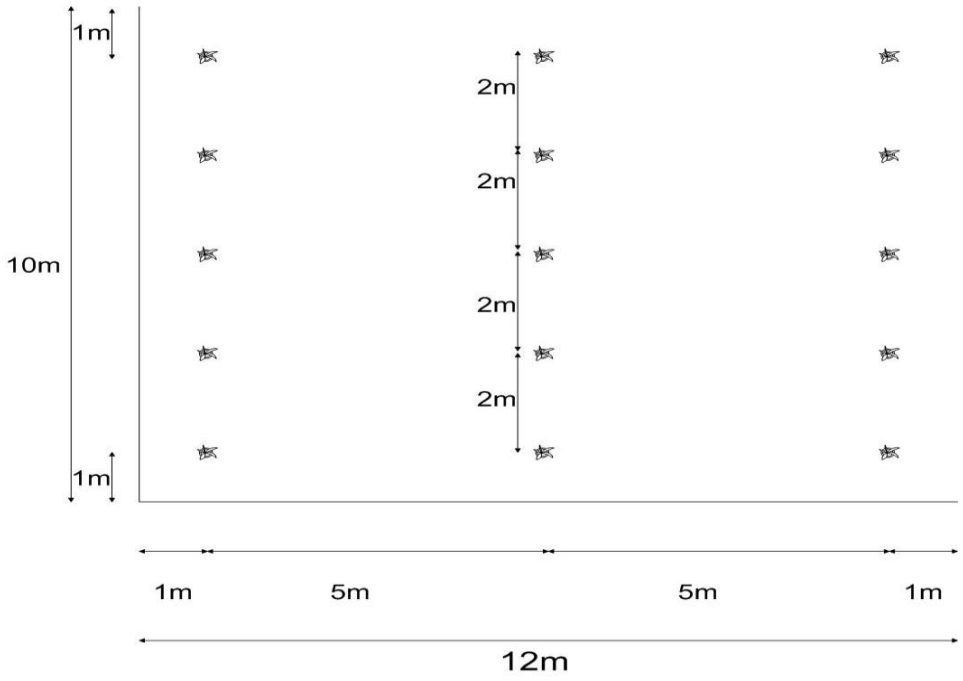


Plate 2.20: Layout of LTS seedlings on the field.

On the entire plot, three columns and five rows were prepared. Five seedlings of each of the tree species: *Bauhinia monandra*, *Delonix regia* and *Tetrapleura tetraptera* investigated in 2013 were randomly selected and planted on 01/10/13 in the prepared holes with each species along a column (**Plate 2.21a, b**). Plant growth and development parameters (tree height, girth (diameter), number of leaf and branches) were taken at an interval of 6 months to determine the survival of the tree species in the field.

Plate 2.21: Establishment of tree species in the field: (a) *Delonix regia* (b) *Bauhinia monandra*.

2.10. Investigation of kaolinite oil-sorption

This section covers the methodology used in the investigation of the potential of kaolinite as a soil pre-treatment for oil-sorption and thus, for spillage remediation. The materials used for the study included: Fume cupboard, Bunsen burner, Büchner funnel, beaker, retort stand and clamp, beaker, measuring cylinder, Whatman filter paper 42, funnel, tropical kaolinite, fresh engine oil (as crude oil was unavailable) and analytical equipment.

Methods:

The amount of engine oil-sorption by the kaolinite was used to determine its possible importance as a soil pre-treatment and amendment in the remediation of oil spillages. To achieve this, two experiments (10.0 g kaolinite + varying amounts of oil; 20.0 g kaolinite + varying amounts of oil) were set up at the laboratory. In the first experiment, 10 g of kaolinite was contaminated with varying amount of engine oil (25, 50, 75, 100 ml) representing the treatments. A control experiment with 0 g, but a

known amount of oil was also set up in each sub-experiment. Each treatment was replicated five times as shown in (**Plate 2.22**).

Plate 2.22: Oil-sorption experiment apparatus.

10.0 g kaolinite was contaminated with varying amount of oil (as sub-experiment) as follows:

a. 10 g + 25 ml oil and replicated 5 times.

b. 10 g + 50 ml oil and replicated 5 times.

c. 10 g + 75 ml oil and replicated 5 times.

d. 10 g + 100 ml oil and replicated 5 times.

0 g + known amount of oil was set up in each sub-experiment as control

A similar experimental set up (as shown above) was adopted for 20.0 g kaolinite and contaminated with varying amounts of oil. In each sub-experiment (a, b, c and d

above), the Büchner funnels were lined with Whatman filter paper 42 and 10.0 g kaolinite was weighed and appropriate volumes of engine oil were placed in the separatory funnel and allowed to dripped overnight through the kaolinite (**Plate 2.22**). The oil was allowed to drip slowly soaking up the kaolinite (**Plate 2.23**) and excess oil was collected in the measuring cylinder situated underneath each Büchner funnel.

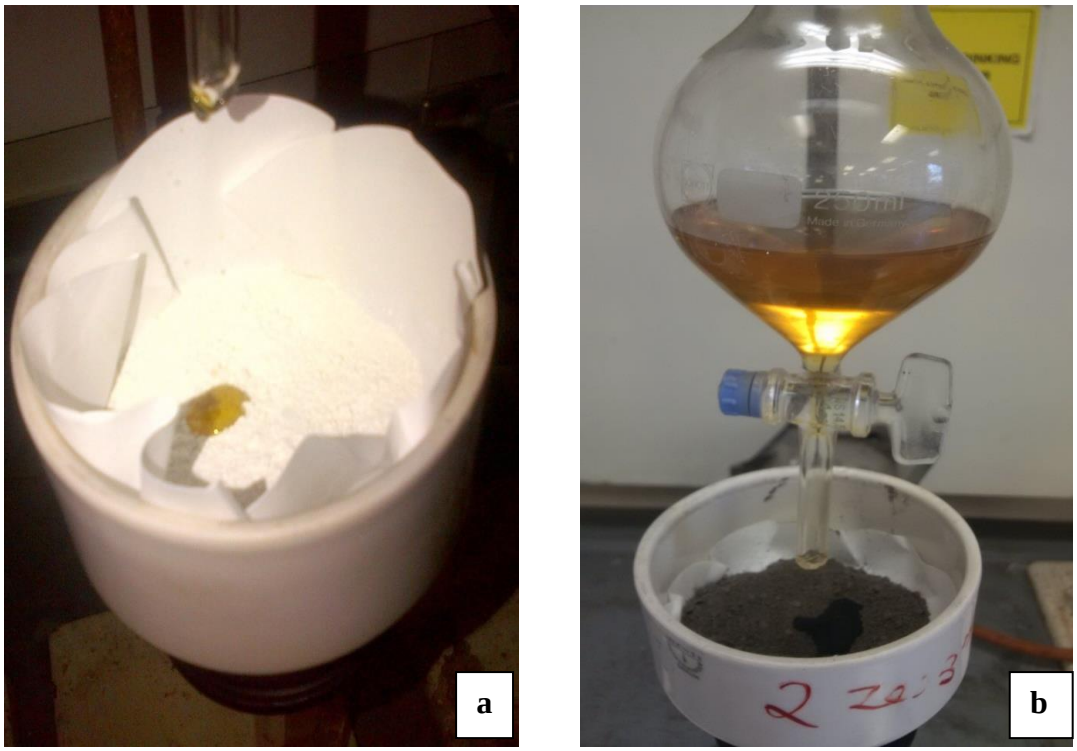


Plate 2.23: Oil-sorption by tropical kaolinite (a) fresh kaolinite (b) burnt kaolinite

The amount of oil collected in the measuring cylinder and amount of oil sorbed by the kaolinite was determined on completion of filtration experiments. The same filtration process was repeated for the 20.0 g kaolinite contaminated with varying amounts of oil and the experiment was replicated five times.

The 100 ml oil-contaminated samples of the 10.0 g and 20.0 g kaolinite were selected for further investigations. It is informative to know that the highest level of

contamination in the present study was 100 ml oil and its concentration in the soil was 2.5 %v/w. The selected samples were placed separately in the cooking pot and the oil completely burnt using the Bunsen burner in the fume cupboard (**Plate 2.24**). The oil-sorbed kaolinite samples were burnt to remove the oil content of the samples and thereafter it re-usability investigated for subsequent oil-sorption. This was achieved in a further experiment using the filtration procedures discussed above.

Plate 2.24: Ignition of oil-sorbed kaolinite for possible re-use for adsorption.

2.11. Analytical studies on the dry fresh and burnt kaolinite samples

Analytical studies were carried out on the dry fresh and burnt kaolinite samples using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD).

2.11.1. Scanning Electron Microscopy (SEM)

SEM reveals the morphology and particle size of crystalline samples. It was found very useful in the kaolinite experiment and was used to compare images of the fresh dry kaolinite before and after it was burnt.

Principle

The principle of SEM uses high energy electrons to form an image. An electron beam is produced at the top of microscope and directed at the specimen to generate various signals at the surface or near surface structure of the selected specimen (Goodhew and Humphereys, 1998). The accelerated beam of electrons scans the specimen by the scan coils and the secondary electron detector of the microscope, which has a positve bias, and detects low energy secondary electrons or other forms of radiation produced from each point on the surface or near-surface of the specimen (Bozzola and Russell, 1999).

Methods

The dry fresh and burnt kaolinite specimens were studied using a SEM (Zeiss Evo 50 fitted with an Oxford EDX, Zeiss, UK). The scanning electron microscopy (**Plate 2.25**) reveals information about chemical composition, crystalline structure and the external morphology of the fresh and burnt kaolinite specimens. A thin layer of each of the specimens was prepared onto aluminium stubs coated with adhesive using a carbon barking pad. Electron images were digitally captured under variable pressure conditions to reduce charging. Images were obtained at magnifications of 10,000 and 5,000 for the specimens of fresh and burnt kaolinite.

Plate 2.25: Scanning Electron Microscopy (SEM)

2.11.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a non-destructive and rapid method that gives accurate results without any external calibration. It was found useful in the study for characterisation and identification of the fresh and burnt kaolinite. FTIR technique uses an infrared absorption spectrum in the identification of kaolinite.

Principle

The working principle of FTIR is that it involves twisting, rotating, bending and vibration of chemical bonds. Kaolinite adsorbs infrared (IR) radiation from the photospectrometer and the extent of adsoption is determined by atomic mass, length, strength and force constant of interatomic bonds in kaolinite structure (Lamberti, 2004). The multiplicity of vibrations occurring simultaneously produces a highly complex absorption spectrum, which is a unique characteristic of the functional groups comprising the molecular structure and configuration of the atoms. A detector

monitors the wavelength range and transmits the signal to a computer which translates the signal into an absorption spectrum.

Method

For the identification of the kaolinite samples, a Genesis II FTIR^{TM} spectrometer (SEMSIR, UK) was used. Small quantities (~0.5 g) of finely dried kaolinite samples were placed onto the scanner of the spectrometer, and the samples were loaded by using a small metal rod to gently apply pressure to the sample. A background scan was first carried out before the sample was scanned to obtain spectra. The FTIR spectrometer uses software called WINFIRST which scanned the samples and produced resulting Infra-Red (IR) spectra with different peaks. The resulting spectrum usually represents the molecular absorption and transmission, thereby producing a molecular fingerprint of the sample. Each spectrum is unique to its own molecular structure and each peak in a spectrum represents a specific bond in the compound. Spectra peaks from the dry fresh and burnt kaolinite samples were compared with the standard peaks of commercial samples of kaolinite for peak identification on the attached PC system.

2.11.3. X-Ray Diffraction (XRD)

XRD is one of the definitive techniques applied in the identification of zeolites and zeolitic materials. It reveals the framework of any sample through its powder using Rietveld refinement techniques (Jentys and Lercher, 2001). The crystallinity and unit cell parameters of a sample can also be determined by XRD.

Principle

The principle of XRD is based on Bragg's law:

$2d\sin\theta = n\lambda$ (Jenkins, 1988) 2(14)

Where:

n = difference in path length between adjacent X-ray beams (order of reflection)

λ = wavelength of the beams (incident X-rays) and is a known value

d = spacing between the crystal planes (inter-planar spacing) and

θ is the angle of scattering (incidence).

XRD is a non-destructive analytical procedure where atomic planes of a crystal make a beam of X-rays scatter at specific angles from each set of lattice planes in a sample. The intensities of the peaks are determined by the arrangement of atoms within the lattice. Regularly distributed atoms within the sample reflect the X-ray beam constructively and results in sharp narrow peaks representing a crystalline sample, while curves emerge as a result of randomly occurring atoms. Whenever a monochromatic X-ray beam with a wavelenghth (λ) is incident on lattice planes on a crystal at an angle (θ), diffraction occurs only when the distance travelled by the rays reflected from successive planes differs by a complete number n of wavelengths. Equations (2.14) can be re-arranged and the angle of diffraction of X-ray radiation relates the given wavelength to the distance between atomic planes of identical type in the unit cell (*d*-spacing) and the incident angle of radiation.

Bragg's law produces a unique lattice spacing for a particular crystalline phase. As a result of Bragg's law, X-ray diffraction from a given atomic plane in the crystal produces a peak at a certain 2θ angle in the diffraction pattern. The most intense peak occur in the range 5º 2θ-40º 2θ for most zeolites (Szostak, 1989).

Method

In the present study, crystallinity of the fresh and burnt kaolinite samples was determined using powder X-Ray Diffraction (XRD) analysis (**Plate 2.26**). The

PAaNalytical empyrean X-ray diffractometer (Philips model PW1770) was used to obtain high quality diffraction data of the kaolinite samples. About 0.2 g of fine powder of kaolinite samples was prepared by grinding in a mortar with pestle. The fine powder was then poured into a standard sample holder using a thin spatula until the sample holder was completely filled to avoid surface irregularities. The sample holder was gently tapped on a table to compact the powder in the holder. The powder was then pressed onto the sample holder using a glass slide to ensure that the top of the sample is in the same plane as the top of the sample holder. Samples were then scanned between 3-50 degrees 2-theta using the PANalytical empyrean X-ray diffractometer (Philips model PW1770) with step width 0.0020 º2θ and a count time of 0.40 seconds per step, to produce the XRD pattern.

Plate 2.26: X-Ray Diffraction (XRD) spectrometry.

2.11.4. X-Ray Fluorescence (XRF)

XRF is employed in the determination of elemental composition of zeolites and zeolitic materials. Dziunikwski (1978) described XRF analysis as a wavelength dispersive X-ray method. XRF technique is capable of detecting and determining each element present in solid or liquid sample from 1 ppm to 100%.

Principle

XRF technique is based on the theory that each individual element produces a unique pattern of fluorescence spectra of specific wavelength. The technique bombards the sample with primary X-rays. This leads to elements fluorescing and generating secondary X-rays, which are thereafter, analyzed using an artificial crystal with known atomic spacing, to determine X-ray wavelengths. Individual elements exhibit this characteristic, being related to the energy wavelengths of different electron shells. The analysis gives data as the oxidised form of each element in % w/w Si:Al as well as any other elements present in the zeolite structural framework.

Method

Samples of fresh and burnt kaolinite fine powder were placed in between the gas permeable membrane (microporous film) and 6 µm X-ray transparent film (Mylar). The samples were then analyzed by the PANanalytical Epsilon 3^{XLE} spectrometer. The fresh and burnt kaolinite samples were analysed for their chemical composition by XRF spectrometry (**Plate 2.27**).

Plate 2.27: X-Ray Fluorescence (XRF) spectrometry.

2.12. Statistical analysis of data

Data obtained from the experiments were analysed using computer package SPSS, version 20 for Windows. Plant Experiments I and II and kaolinite experiment were treated separately using a One-way Analysis of Variance (ANOVA). All data were tested for homogeneity of variance, to determine their suitability for ANOVA tests before analysis. Data were also analysed for least significant differences (LSD) within the treatments for each experiment separately. Correlation analysis (r) between contamination and plant growth was also determined. Data means were separated and compared using Duncan's Multiple Range test at $P < 0.05$.

RESULTS

The results of **Plant Experiment I**, in which *Bauhinia monandra*, *Delonix regia* and *Tetrapleura tetraptera* were investigated 2013 and **Plant Experiment II**, in which *Albizia adianthifolia*, *Albizia odoratissima* and *Pterophorum pterocarpum* were studied in 2014, are reported in **Chapters 3** and **Chapter 4**, respectively. In each chapter, reports were made on the % germination of the selected tree species, plants' early growth performance in the greenhouse, plant biomass production, nodulation, physicochemical and microbial properties of soils (un-contaminated, contaminated and remediated), total petroleum hydrocarbon degradation in the rhizosphere and establishment of seedlings of the investigated tree species in the field. **Chapter 5** reports on the oil sorption potential of kaolinite and its possible role in the remediation of oil spills.

CHAPTER THREE

Results of Plant Experiment I

3.0. Introduction

In this chapter, experimental data along with the statistical analyses for the three 'Leguminous Tree Species' (LTS) grown in crude oil-contaminated soil in 2013 are presented. These LTS are *Bauhinia monandra*, *Delonix regia* and *Tetrapleura tetraptera*. The focus of the study are germination of the selected tree species in crude oil-contaminated soil, early growth performance in the greenhouse, plant biomass production, nodulation, physicochemical and microbial properties of the soil on which the LTS were grown, hydrocarbon degradation and establishment of seedlings of the LTS tree species in the field. The results obtained from the investigation of these LTS are reported under the following headings.

3.1. Percentage germination of LTS in crude oil-contaminated soil water extracts

The ability of plant species to germinate in crude oil-contaminated soil is the first step in determining the tolerance of the species to soil conditions. The percentage seed germination and Co-efficient of Velocity (COV) of germination of the selected LTS moistened with varying crude oil-contaminated soil water extracts is presented **Table 3.1**. The ability of seeds of the selected LTS to germinate varied greatly. It was observed that seed germination and COV of germination of the LTS studied were directly proportional to contamination as the COV observed in *D. regia* accords with its good germination rate, while the poor COV observed in *T. tetraptera* also corresponds with its germination rate.

Table 3.1: Percentage seed germination and co-efficient of velocity of germination of selected LTS in crude oil-contaminated water extracts

Treatment (ml)	*D. regia*		*B. monandra*		*T. tetraptera*	
	Gt* (%)	CoV** (%)	Gt* (%)	CoV** (%)	Gt* (%)	CoV** (%)
0	100	69.80	100	68.12	88	65.08
25	90	64.49	90	66.46	80	64.06
50	82	61.85	74	65.50	72	64.09
75	78	62.48	70	64.10	68	64.09
100	60	60.99	58	61.95	56	62.02

*Germination (%)

**Co-efficient of Velocity (%).

Seed germination is a good determinant of plant growth in the medium on which it is grown. Seeds of the selected LTS germinated at different rates (**Table 3.1**) due to many factors ranging from nature of the seed to the prevailing environmental conditions in the growth medium. **Figure 3.1** shows the germination percentage of *D. regia* in crude oil contaminated soil water extracts. Soil treated with 0, 25, 50, 75 and 100 ml crude oil had 100, 90, 82, 78 and 60% germination, respectively. % Germination of *D. regia* seeds in the various level of contamination (treatments) were significantly different ($P < 0.05$) by one-way ANOVA (**Appendix 3.1**). The mean comparison by LSD among the treatments also revealed that there were significant differences ($P < 0.05$) in seed germination at the various contamination levels. *D. regia* seed germination and contamination were strongly correlated ($R^2 = -0.976$; $n = 5$; $P < 0.05$) (**Appendix 3.2**).

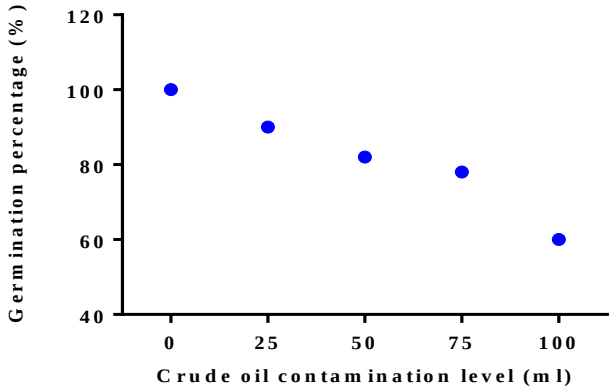
Germination of 100, 90, 74, 70 and 58 % were achieved in *B. monandra* moistened with the varying crude oil-contaminated water extracts (**Figure 3.2**) and there were significant differences at ($P < 0.05$) by one-way ANOVA (**Appendix 3.3**) when mean

of seed germination were compared using LSD. The rate of germination also strongly correlated (R^2 = -0.988; n = 5; P <0.05) with the level of contamination (**Appendix 3.2).**

T. tetraptera had a germination success of 88, 80, 72, 68 and 56% when moistened with 0, 25, 50, 75 and 100 ml crude oil contaminated soil water extracts, respectively (**Figure 3.3**) and there were significant differences (P ≤0.05) (**Appendix 3.4**). The rate of germination was also strongly correlated (R^2 = -0.976; n = 5; P <0.05) with crude oil contamination (**Appendix 3.2)**. It was observed that all these LTS germinated when moistened with crude oil contaminated water extracts, but germination was concentration dependent, as percentage germination decreased with increased oil concentrations in the extracts. Although, the germination percentage of the selected tree species varied, all LTS were observed to tolerate the contaminated condition and germinated. However, *D. regia* tended to have better germination than *B. monandra* and *T. tetraptera*, even at higher concentrations of crude oil (**Figure 3.4**) and there was an inverse association between the germination of the plant species and contamination, at (P <0.05) (**Appendix 3.2**). One-way ANOVA between the mean germination of the tested plant species and the level of contamination shows that there were significant differences among the mean germination of the LTS at the contamination levels (P <0.05). It was also revealed that there were no significant differences between the germination of *D. regia* and *B. monandra*, but there was a significant difference between *D. regia* and *B. monandra* when compared with *T. tetraptera* (P <0.05) (**Appendix 3.5**). The level of contamination in the LTS also influenced the co-efficient of velocity of germination (COV) (**Table 3.1**), and the speed of germination was significantly different (P <0.05). Germination

percentage was influenced by the amount of oil in soil and therefore germination was concentration dependent. The complimentary results obtained between percentage germination and COV support this assertion (**Appendix 3.6, Table 3.2**). These tested tree species particularly, *D. regia*, therefore tends to hold promise for phytoremediation of crude oil contaminated soil and re-vegetation of such soils.

Contamination level (ml)	Germination percentage (±SD) of 5 replicates
0	100.00±0.00
25	90.00±0.45
50	82.00±0.49
75	78.00±0.37
100	60.00±0.32

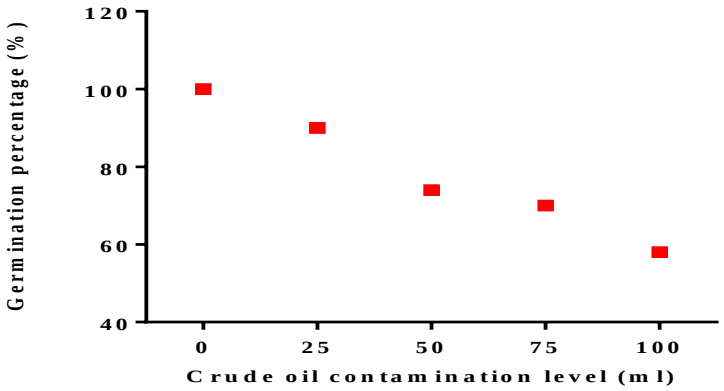


n = 5; N = 25; r = -0.976; P <0.004*; *Significant at P<0.05.

±SD = Standard deviation.

Figure 3.1: Germination of *D. regia* in crude oil contaminated soil water extracts.

Contamination level (ml)	Germination percentage (±SD) of 5 replicates
0	100.00±0.00
25	90.00±0.45
50	74.00±0.40
75	70.00±0.32
100	58.00±0.49

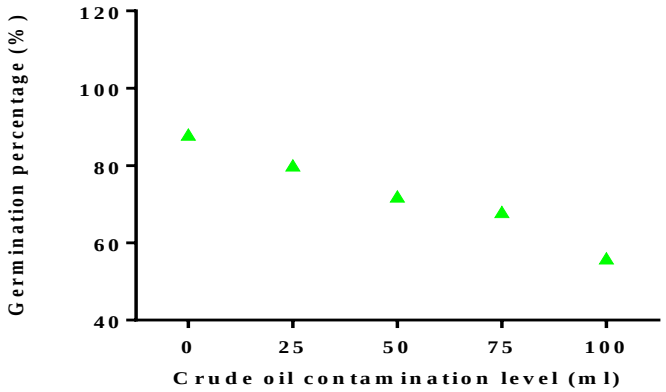


n = 5; N = 25; r = -0.988; P <0.002*; *Significant at P <0.05.

±SD = Standard deviation.

Figure 3.2: Germination of *B. monandra* in crude oil contaminated soil water extracts.

Contamination level	Germination percentage (±SD) of 5 replicates
0	88.00±0.37
25	80.00±0.32
50	72.00±0.37
75	68.00±0.20
100	56.00±0.51



n = 5; N = 25; r = -0.990; P = 0.001* *Significant at P<0.05.

±SD = Standard deviation.

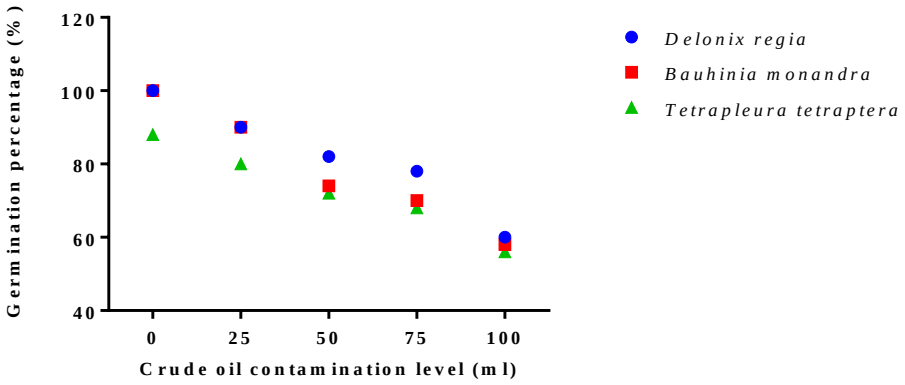
Figure 3.3: Germination of *T. tetraptera* in crude oil contaminated soil water extracts

Germination of LTS*COCL[++]	n	r	Probability P	Decision P <0.05	Conclusion
Delonix regia	5	-0.976	0.004[*]	Significant	Negative[+]
Bauhinia monandra	5	-0.988	0.002[*]	Significant	Negative[+]
Tetrapleura tetraptera	5	-0.990	0.001[*]	Significant	Negative[+]

[+]Negative indicates inverse association between germination of plant species and contamination.

[*]Significant at P <0.05.

[++]Crude oil contamination level



±SD = Standard deviation.

Figure 3.4: Comparison of percentage germination among selected LTS moistened with varying concentration of crude oil contaminated water extracts

Table 3.2: Correlation coefficients betweem LTS and crude oil-contamination

LTS*LoC[†]	Probability r	n	P	P<0.05
D. regia	-0.976	5	0.001[*]	
B. monandra	-0.988	5	0.001[*]	
T. tetraptera	-0.990	5	0.001[*]	

[†]Level of Contamination (LoC).

3.2. Evaluation of early growth performance of the selected LTS in crude oil-contaminated soils

The growth performance of the selected LTS in crude oil-contaminated soil was determined using the growth parameters: plant height, plant girth and number of leaves produced by the LTS during the 16-week period of study. **Figure 3.5** shows the mean height of the selected LTS planted in crude oil-contaminated soils at 2 WAP. The mean height, girth and number of leaves observed in *D. regia* planted in non-contaminated soil were 7.72±6.11, 0.08±0.04 and 2.00±1.14 cm, respectively (**Appendix 3.7**). *B. monandra* had mean height 0.76±1.70 cm and mean girth 0.02±0.04 cm in the control experiment, but there was no growth in the treatments (**Appendix 3.8**). The contaminated soil on which the LTS were planted may have delayed germination and growth. *T. tetraptera* recorded a mean height of 1.30±0.84, 1.20±0.76, 0.76±0.77, 0.24±0.54 and 0.00±0.00 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean girth was 0.08±0.04, 0.08±0.04, 0.06±0.05, 0.02±0.04 and 0.00±0.00 cm, respectively. Only *T. tetraptera* produced leaves in the contaminated soils at 2 WAP (**Appendix 3.9)**.

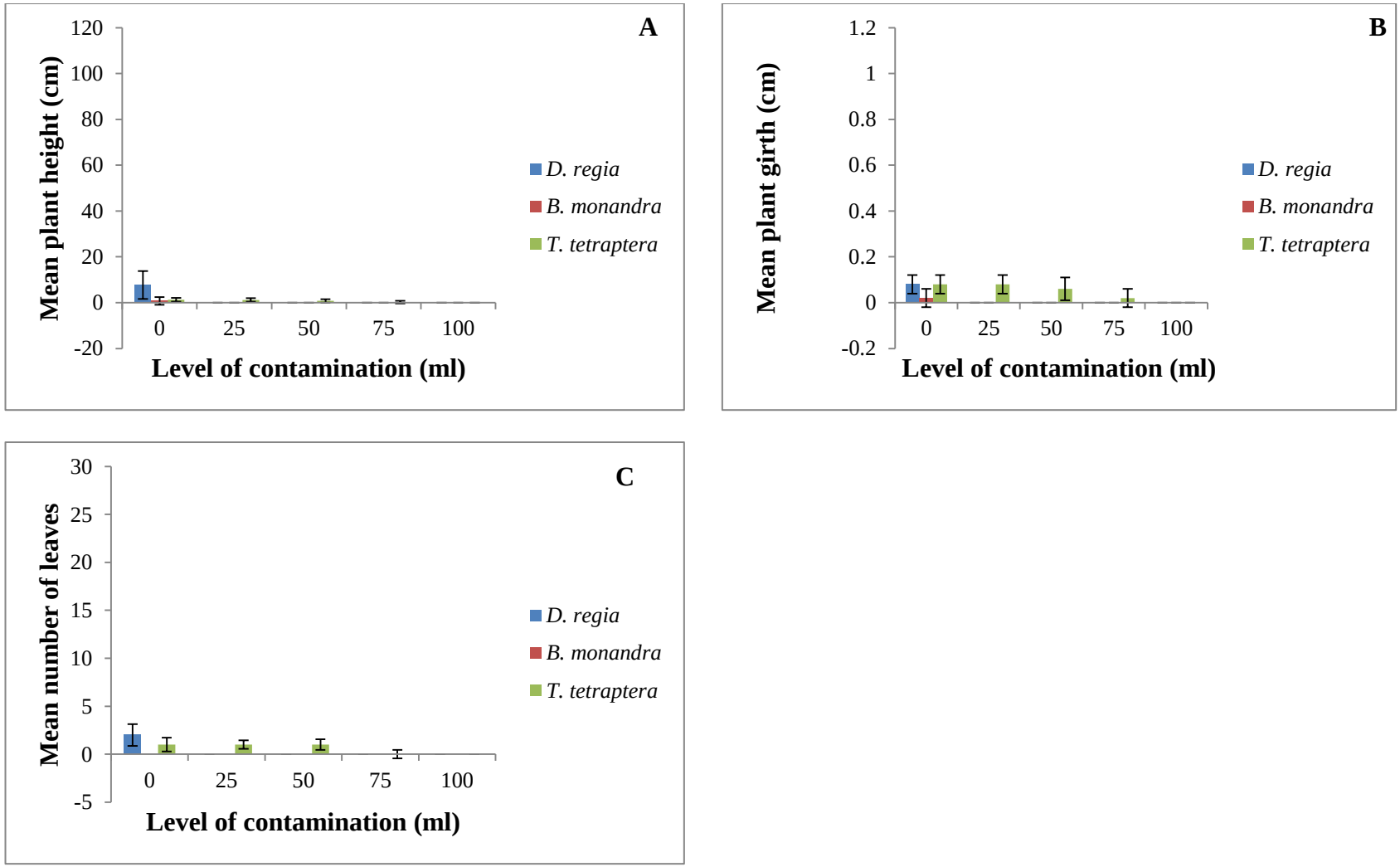


Figure 3.5: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 2 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

Figure 3.6 shows the growth performance of the LTS in crude oil-contaminated soil at 4 WAP. *D. regia* had mean height 22.30±6.58, 2.90±3.04, 1.46±2.02, 0.40±0.89 and 0.00±0.00 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean girth was 0.16±0.05, 0.06±0.05 and 0.04±0.05 cm in treatments 0, 25 and 50 ml oil contaminated soils, but there were no visible plant girth in 75 and 100 ml treatments. Two leaves were produced only in the control experiment (**Appendix 3.10)**. *B. monandra* had mean height 8.50±2.93 and 1.22±2.73 cm in 0 and 25 ml and mean girth 0.12±0.04 and 0.02±0.04 cm in 0 and 25 ml, but there was no growth in 50, 75 and 100 ml treatments (**Appendix 3.11)**. *T. tetraptera* recorded mean height 2.46±0.48, 2.30±0.55, 1.86±0.57, 0.80±1.13 and 0.00±0.67 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. Mean girth of 0.10±0.00 cm was observed in 0, 25 and 50 treatments. Only *T. tetraptera* produced leaves in both the un-contaminated and contaminated soils at 4 WAP (**Appendix 3.12)**. The selected plant species had low growth rates in the first 4 weeks after planting.

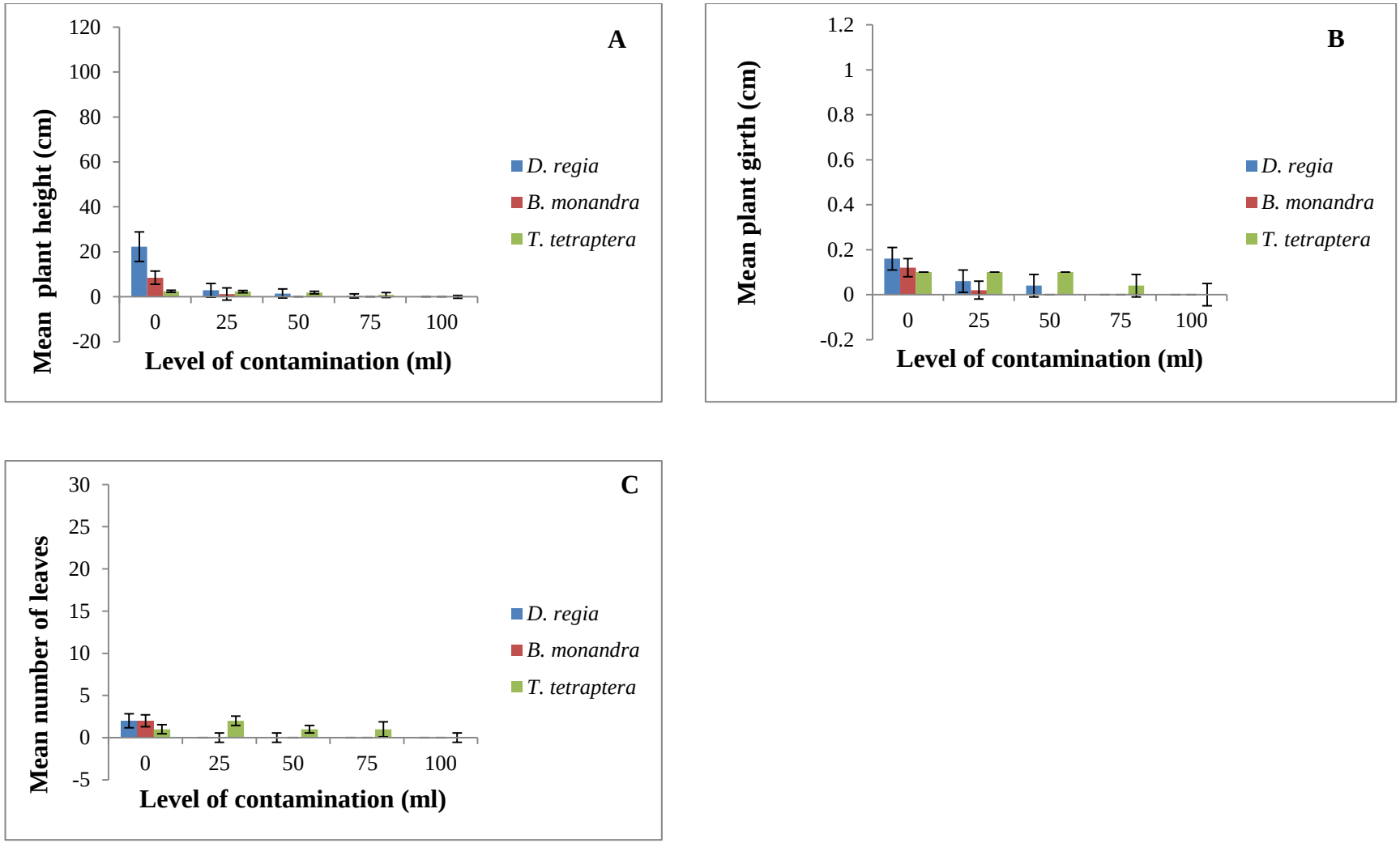


Figure 3.6: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 4 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The growth performance of the selected LTS in crude oil-contaminated soil at 6 WAP is shown in **Figure 3.7**. *D. regia* had mean heights of 39.10±7.39, 15.82±8.50, 12.78±4.51, 5.52±4.93 and 4.02±0.74 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. Mean girths were 0.34±0.11, 0.16±0.05, 0.14±0.05, 0.08±0.00 and 0.01±0.00 cm in treatments 0, 25, 50, 75 and 100 ml oil contaminated soils, respectively. The number of leaves produced was 4.00±1.14, 3.00±1.17, 2.00±0.84, 1.00±0.50 and 1.00±0.00 leaves in the 0, 25, 50, 75 and 100 ml treatments, respectively (**Appendix 3.13)**. *B. monandra* had mean heights of 21.70±3.74, 10.00±4.31, 1.36±1.98, 0.66±1.01, 0.31±0.93 cm, with corresponding mean girths of 0.22±0.04, 0.12±0.04, 0.04±0.00, 0.04±0.05, 0.02±0.05 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. *B. monandra* produced 5.00±1.14 and 2.00±0.71 leaves in 0 and 25 ml crude oil contaminated soils respectively (**Appendix 3.14)**. *T. tetraptera* recorded mean height of 5.88±0.34, 4.84±0.55, 4.40±0.75, 3.08±1.19 and 2.98±1.08 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. Mean girth of 0.10±0.00 cm was observed in all the treatments. Only *T. tetraptera* produced leaves, 5, 4, 3 2 and 2 leaves in 0, 25, 50, 75 and 100 ml crude oil contaminated soils, respectively at 6 WAP (**Appendix 3.15)**.

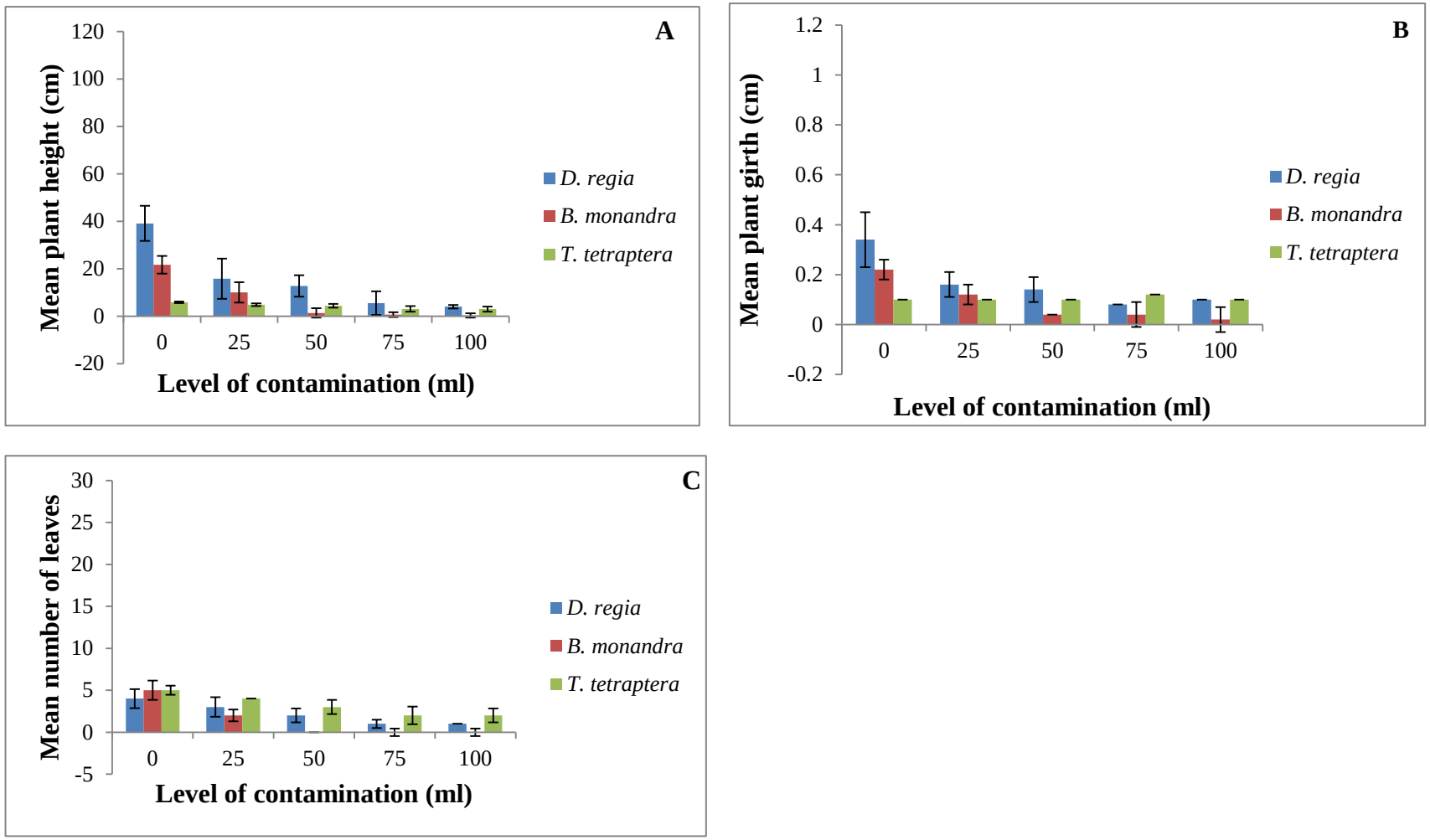


Figure 3.7: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 6 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

Figure 3.8 shows the mean growth performance of the selected LTS in crude oil-contaminated soil at 8 WAP. *D. regia* had mean plant height of 55.00±7.89, 28.94±8.07, 26.38±4.57, 20.32±6.47 and 7.69±1.15 cm. The mean plant girth was 0.46±0.11, 0.24±0.05, 0.24±0.05, 0.20±0.07 and 0.50±0.00 cm while 8.00±1.34, 5.00±1.30, 4.00±1.00, 3.00±0.71 and 1.00±0.00 mean number of leaves were produced in *D. regia* planted in 0, 25, 50, 75 and 100 ml crude oil contaminated soils (**Appendix 3.16)**. In *B. monandra*, mean plant height of 34.54±4.34, 23.80±5.75, 12.72±2.73, 10.44±2.31 and 8.82±4.35 cm were observed in 0, 25, 50, 75 and 100 ml crude oil contaminated soil respectively. The girths were 0.36±0.05, 0.26±0.05, 0.16±0.05, 0.12±0.04 and 0.10±0.00 cm respectively. The number of leaves produced by the tree species increased to 7.00±1.58, 4.00±1.00, 2.00±0.55, 2.00±0.55 and 1.00±0.45 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil respectively (**Appendix 3.17**). *T. tetraptera* had mean plant height of 10.00±0.59, 7.48±1.37, 7.36±1.44, 6.18±1.35 and 5.28±0.68 cm. Mean girth were 0.20±0.00, 0.12±0.04, 0.12±0.04, 0.10±0.00 and 0.10±0.00 cm. It produced 6.00±0.71, 4.00±0.55, 4.00±0.89, 3.00±0.89 and 2.00±0.89 mean leaves in 0, 25, 50, 75 and 100 ml respectively (**Appendix 3.18**).

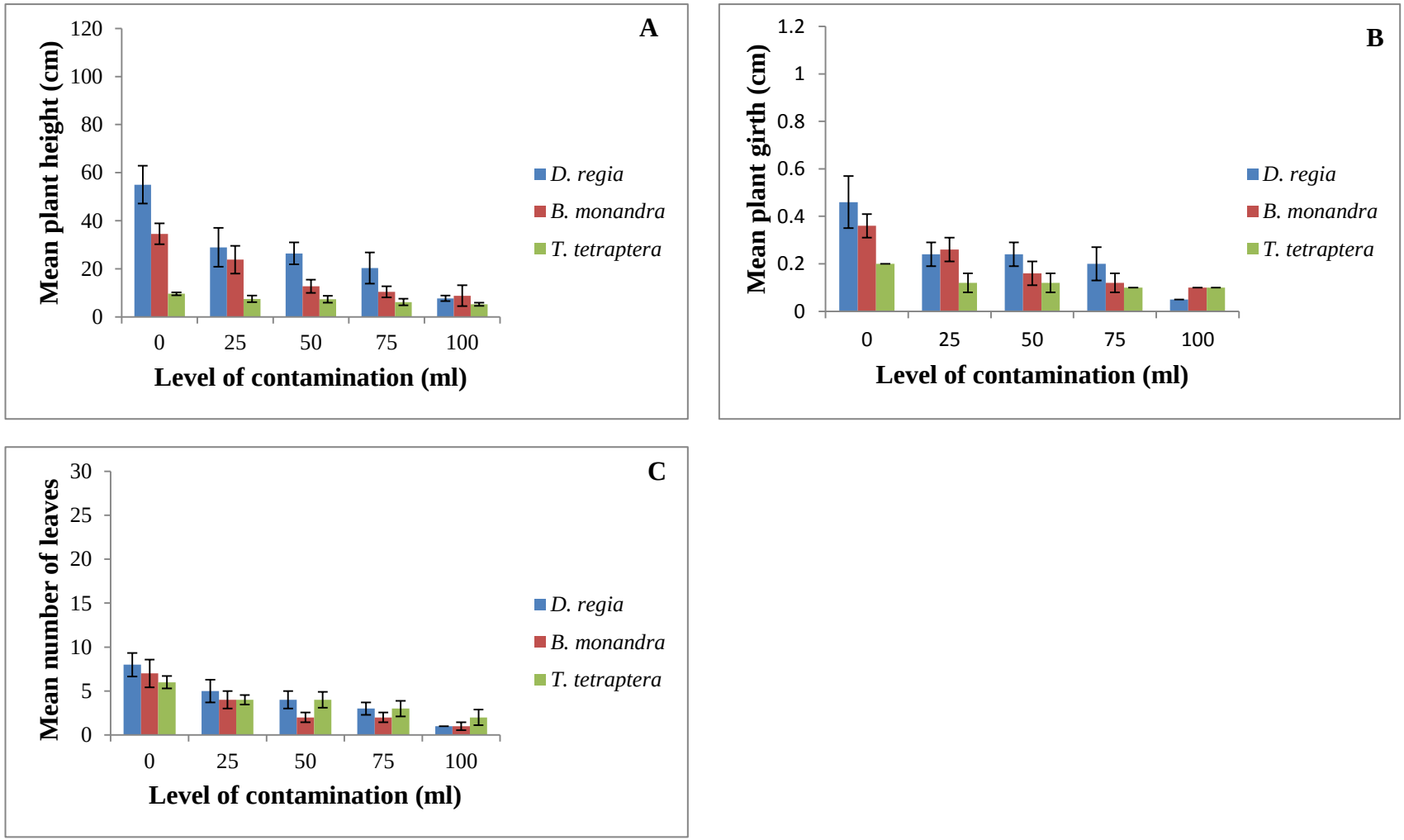
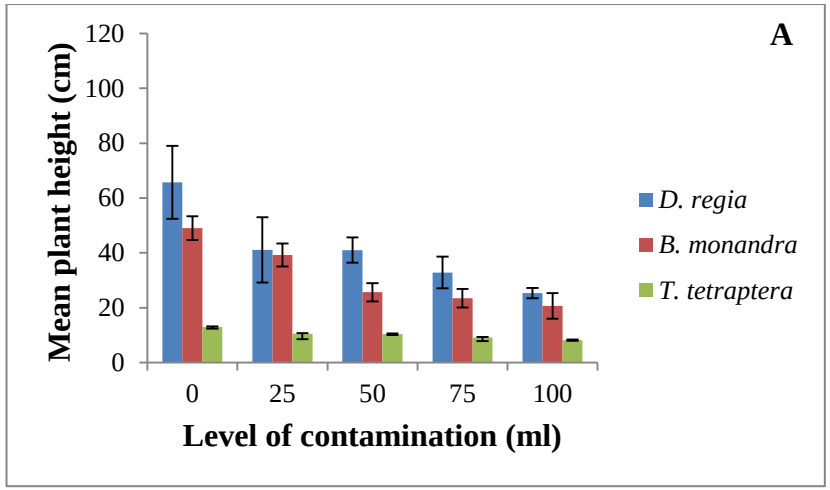
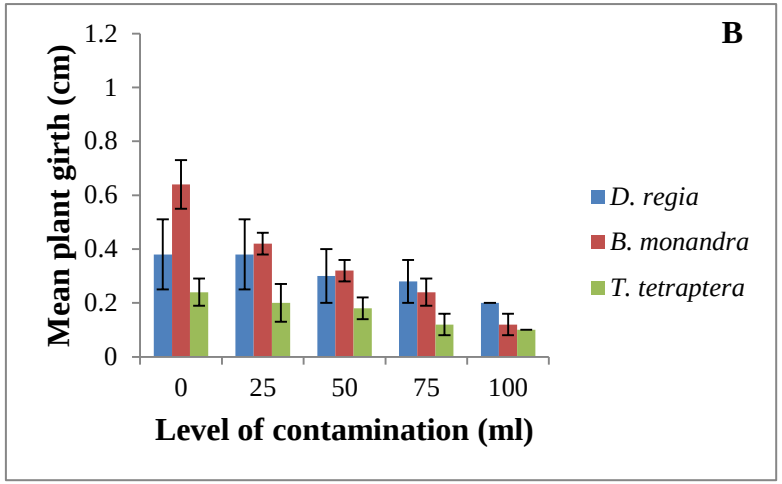


Figure 3.8: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 8 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

Figure 3.9 shows the mean growth performance of the selected LTS in crude oil-contaminated soil at 10 WAP. *D. regia* mean plant height increased to 65.70±13.27, 41.08±11.86, 41.04±4.62, 32.86±5.76 and 25.36±1.88 cm. Mean girth also increased to 0.38±0.13, 0.38±0.13, 0.30±0.10, 0.28±0.08 and 0.20±0.00 cm. The mean number of leaves produced was 13.00±1.92, 9.00±3.67, 7.00±1.10, 5.00±1.34 and 4.00±0.45 in 0, 25, 50, 75 and 100 ml crude oil contaminated soils (**Appendix 3.19)**. *B. monandra* had mean plant height of 49.02±4.32, 39.24±4.17, 25.68±3.31, 23.46±3.38 and 20.72±4.67 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. Mean girths were 0.64±0.09, 0.42±0.04, 0.32±0.14, 0.24±0.05 and 0.12 ±0.04 cm, respectively. The mean number of leaves produced by the tree species increased to 9.00±1.30, 6.00±0.84, 4.00±0.84, 3.00±0.55 and 3.00±0.55 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively (**Appendix 3.20**). *T. tetraptera* had mean plant height of 13.04±0.63, 10.56±2.03, 10.40±0.29, 9.16±1.33 and 8.20±0.23 cm. Mean girths were 0.24±0.05, 0.20±0.07, 0.18±0.04, 0.12 ±0.04 and 0.10±0.00 cm. It produced 8.00±0.89, 5.00±1.58, 5.00±0.45, 3.00±1.00 and 3.00±0.55 mean number leaves in 0, 25, 50, 75 and 100 ml respectively (**Appendix 3.21**). Thus, mean growth of LTS was related to the concentration of oil in soil (**Figure 3.9**) and there was increased growth parameters as the experiment progressed.





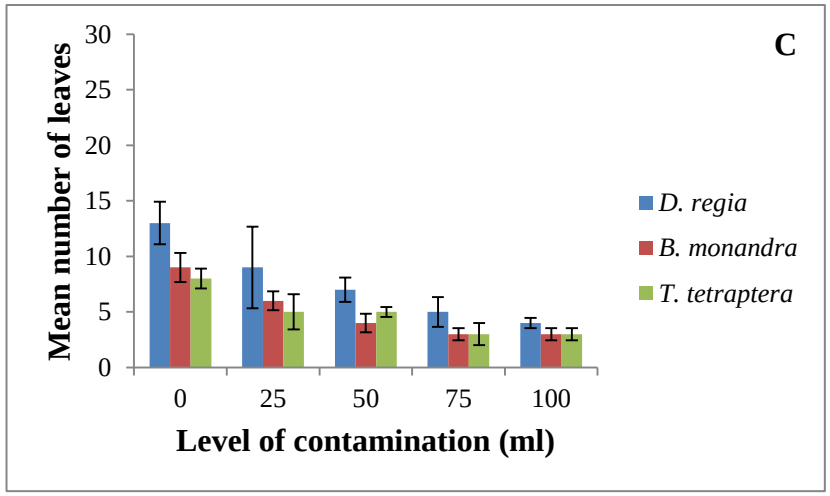


Figure 3.9: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 10 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The mean growth performance of the LTS in crude oil-contaminated soil at 12 WAP is presented in **Figure 3.10**. *D. regia* mean plant height increased to 84.12±8.31, 58.12±6.77, 51.76±4.90, 43.90±3.93 and 35.04±1.77 cm. Mean girth also increased to 0.78±0.08, 0.60±0.00, 0.46±0.05, 0.36±0.05 and 0.26±0.05 cm. The mean number of leaves was 17.00±1.67, 13.00±1.34, 9.00±1.30, 6.00±0.84 and 4.00±0.55 in 0, 25, 50, 75 and 100 ml crude oil contaminated soils (**Appendix 3.22)**. *B. monandra* had mean plant heights of 60.54±5.50, 51.60±4.67, 37.98±2.76, 33.84±3.54 and 30.48±3.83 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. Mean girths were 0.70±0.14, 0.48±0.08, 0.24±0.13, 0.30±0.00 and 0.16±0.05 cm, respectively. Mean number of leaves produced by the trees increased to 10.00±1.79, 7.00±0.84, 4.00±0.89, 3.00±0.45 and 4.00±0.89 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively (**Appendix 3.23**). *T. tetraptera* had mean plant heights of 15.64±0.83, 13.00±2.08, 13.26±0.62, 11.62±0.97 and 10.66±0.65 cm. Mean girths were 0.40±0.00, 0.20±0.05, 0.28±0.04, 0.20±0.00 and 0.16±0.05 cm. mean number of leaves were 8.00±0.45, 7.00±1.52, 6.00±0.71, 4.00±0.84 and 3.00±0.00 in 0, 25, 50, 75 and 100 ml, respectively (**Appendix 3.24**). Thus, the mean growth of the trees was strongly influenced by concentration of oil in soil.

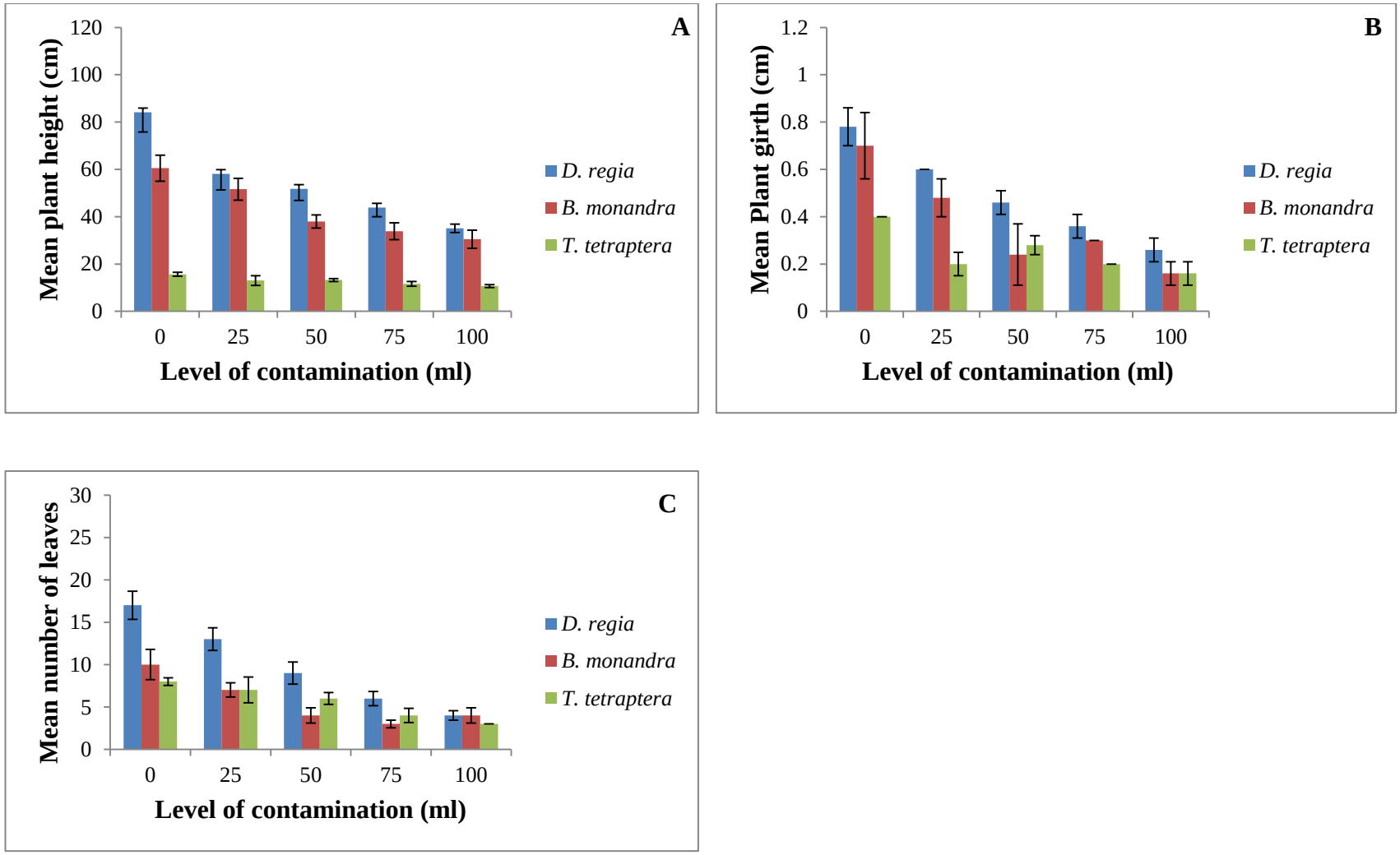


Figure 3.10: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 12 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The mean growth performance of the selected LTS in crude oil-contaminated soil at 14 WAP is shown in **Figure 3.11**. The mean plant height of *D. regia* planted in crude oil contaminated soil was 98.40±13.16, 70.50±6.40, 64.62±5.04, 54.64±4.84 and 45.14±1.24 cm. Mean girth was 0.88±0.11, 0.74±0.05, 0.58±0.04, 0.44±0.09 and 0.30±0.00 cm, while the mean number of leaves produced was 18.00±2.45, 14.00±1.64, 11.00±2.41, 8.00±1.14 and 5.00±0.45 in 0, 25, 50, 75 and 100 ml crude oil contaminated soils (**Appendix 3.25)**. *B. monandra* had mean plant height of 75.90±3.67, 63.04±4.64, 49.66±4.02, 43.54±4.51 and 41.18±4.33 cm. The mean girths of the seedlings were 0.78±0.04, 0.60±0.07, 0.42±0.05, 0.38±0.08 and 0.30±0.10 cm, while the mean number of leaves increased to 12.00±1.30, 8.00±0.89, 5.00±0.45, 4.00±1.30 and 4.00±0.89 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil respectively, (**Appendix 3.26**). *T. tetraptera* had mean plant height of 18.36±1.34, 15.38±1.97, 15.06±0.66, 13.64±1.06 and 11.94±0.40 cm. Mean girths were 0.38±0.04, 0.28±0.04, 0.30±0.00, 0.20±0.00 and 0.14±0.05 cm, while its leaf production increased to 8.00±1.10, 7.00±1.10, 6.00±1.14, 4.00±0.84 and 3.00±0.00 in 0, 25, 50, 75 and 100 ml, respectively (**Appendix 3.27**).

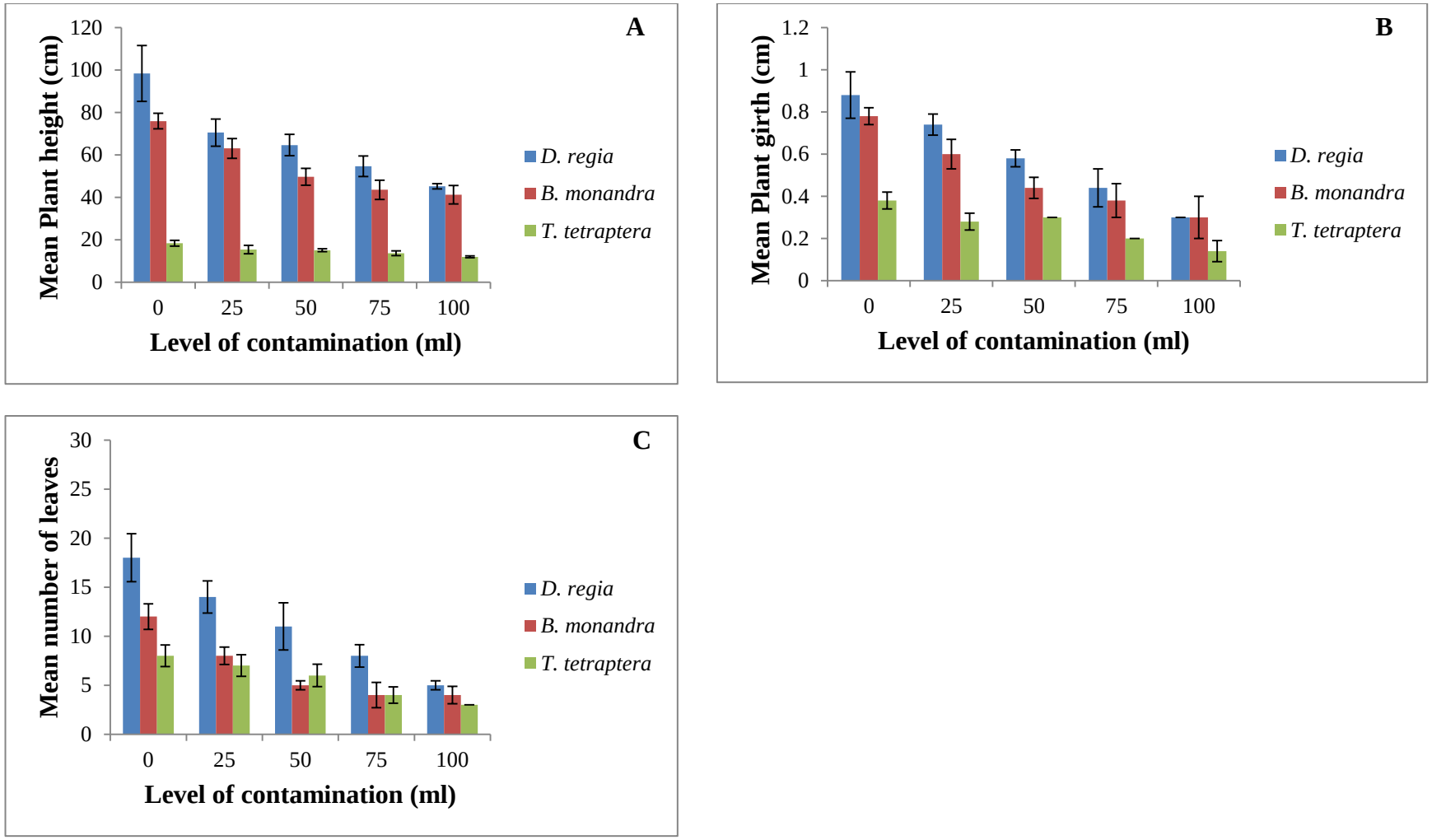
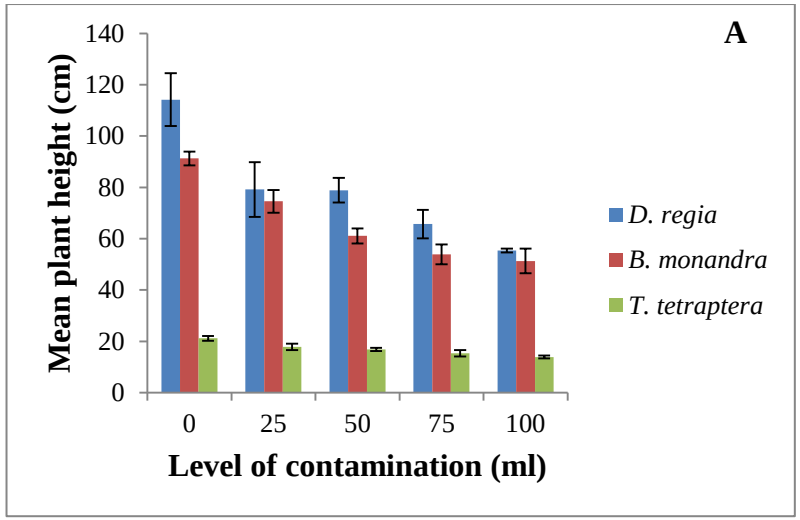
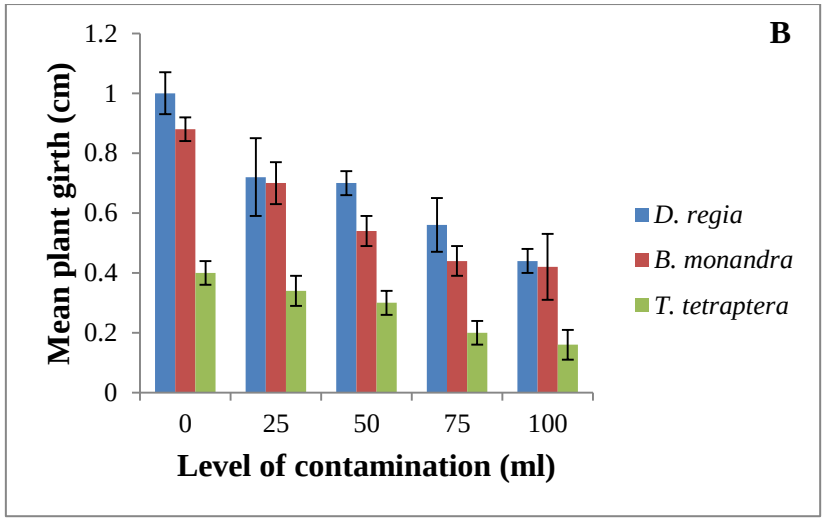


Figure 3.11: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminate soil at 14 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The 16 WAP marked the end of the early growth study and the mean growth performance of the selected LTS is as shown in **Figure 3.12**. *D. regia* had mean height 114.20±10.34, 79.18±10.68, 78.90±4.84, 65.68±5.54 and 55.36±0.78 cm. Mean girths were 1.10±0.07, 0.72±0.13, 0.70±0.04, 0.56±0.09 and 0.44±0.04 cm, while the mean number of leaves was 23.00±1.73, 15.00±3.58, 13.00±1.87, 10.00±1.52 and 6.00±0.71 in 0, 25, 50, 75 and 100 ml crude oil contaminated soils (**Appendix 3.28)**. *B. monandra* had mean plant height 91.26±2.66, 74.58±4.44, 61.10±2.92, 53.88±3.90 and 51.32±4.78 cm. Mean girths were 0.88±0.04, 0.70±0.07, 0.54±0.05, 0.44±0.05 and 0.42±0.11 cm, while the mean number of leaves increased to 14.00±1.52, 9.00±0.89, 6.00±0.84, 6.00±0.89 and 4.00±0.89 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively (**Appendix 3.29**). *T. tetraptera* had mean plant height of 21.16±0.97, 17.86±1.24, 16.80±0.62, 15.34±1.23 and 13.90±0.58 cm. Mean girth was 0.40±0.04, 0.34±0.05, 0.30±0.04, 0.20±0.04 and 0.16±0.05 cm, while leaf production increased to 9.00±0.55, 7.00±1.00, 7.00±0.84, 5.00±1.14 and 3.00±0.55 in 0, 25, 50, 75 and 100 ml, respectively (**Appendix 3.30**). The overall results showed that the mean growth of the trees is related to the concentration of oil in soil and there continues to be an increase in the studied growth parameters with time.





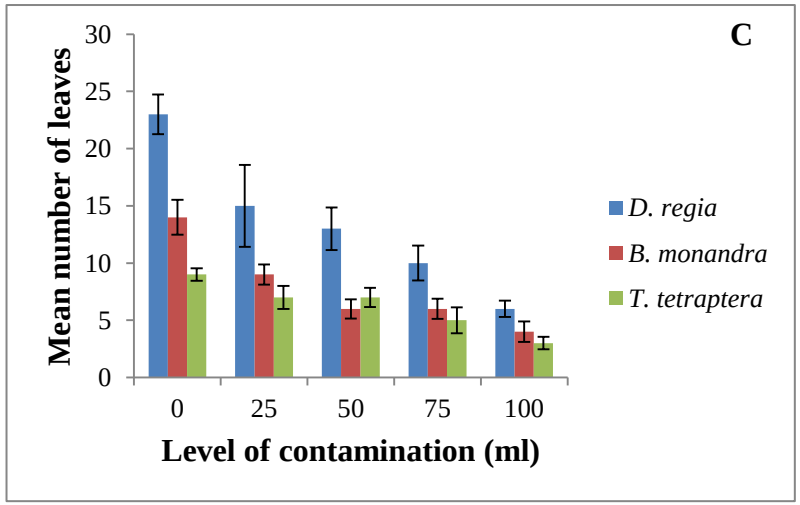


Figure 3.12: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 16 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

One-way ANOVA and means separated by LSD at P <0.05 of growth parameters (height, girth and number of leaves) at 16 WAP revealed that the growth parameters of all species were significantly different at the various levels of contamination from those grown in non-contaminated soil. Pearson correlation test between seedling heights and contamination shows that the heights of *D. regia* were strongly negatively correlated (R^2 = -0.885; n = 25; P <0.01) (**Appendix 3.31**) and this attests to its good germination reported earlier. Similarly, the girth of *D. regia* were strongly correlated (R^2 = -0.898; n = 25; P <0.01) (**Appendix 3.32**) with the girth of the two other species. Leaf numbers were also strongly correlated with contamination (R^2 = -0.898; n = 25; P <0.01) (**Appendix 3.33**).

3.3. Nodule production in the selected LTS

Nodule production is one of the peculiar features of legumes. Microbes in the rhizosphere inhabit the nodules and in turn offer some advantageous effects to the plants. **Table 3.3** shows the number of nodules produced by the leguminous tree species grown in the crude oil-contaminated soils in the greenhouse. *D. regia* produced a mean of 19, 17, 15, 15 and 10 nodules in 0, 25, 50, 75 and 100 ml crude oil-contaminated soil, respectively. Nodule production in *B. monandra* was similar to that of D. *regia* and the nodules share some physical resemblance. *B. monandra* produced 17, 15, 11, 8 and 6 nodules in the 0, 25, 50, 75 and 100 ml crude oil-treated soil, respectively. However, nodule production in *T. tetraptera* was considerably less than the first two tree species. A total of 12, 12, 9, 4 and 2 nodules were observed in 0, 25, 50, 75 and 100 ml treated soil, respectively. *T. tetraptera* had low nodule production in the high crude oil-contaminated soil. The overall results of nodule production among the tree species revealed that the number of nodules produced

decreased as the concentration of crude oil in the soil increased. This may indicate that the soil microbes can only survive in low crude oil-contaminated soil. A typical nodule produced by the leguminous tree species is shown in **Figure 3.13**.

Figure 3.13: Typical nodule production among LTS grown in crude oil-contaminated soil.

Table 3.3: Mean nodules produced by the LTS grown in crude oil-contaminated soil

LTS/LoC*	**Mean number of nodules**				
	0	**25**	**50**	**75**	**100**
D. regia	19	17	15	15	10
B. monandra	17	15	11	8	6
T. tetraptera	12	12	9	4	2

*LoC = Level of Contamination (ml).

3.4. Shoot and root biomass of the selected LTS grown in crude oil-contaminated soil

Results of shoot and root biomass (dry-matter content) of the LTS grown in crude oil-contaminated soil show that *D. regia* had mean shoot and root biomass of 107.73±12.91 g and 26.50±1.95 g, respectively after 16 weeks of growth. The seedlings grown in crude oil-contaminated soil had mean shoot biomass of 71.37±3.16, 47.80±3.22, 41.90±1.55, 38.97±8.31 g and the root systems of these seedlings produced mean biomass of 11.27±2.86, 7.37±1.06, 6.87±1.16 and 6.50±0.61 g, respectively, in 25, 50, 75 and 100 ml oil-contaminated soil (**Table 3.4**). The seedlings of *B. monandra* produced mean shoot biomass of 49.00±4.89, 49.93±7.13, 26.10±9.79, 9.87±1.89 and 5.67±1.22 g with a corresponding mean root biomass of 12.23±1.98, 11.30±0.78, 5.50±0.82, 2.27±0.42 and 1.70±0.40 g when grown in 0, 25, 50, 75, 100 ml crude oil-contaminated soil (**Table 3.4**). Similarly, seedlings of *T. tetraptera* grown on crude oil-contaminated soil produced mean shoot biomass of 8.50±0.82, 6.70±0.70, 5.90±1.15, 2.40±1.25 and 1.13±0.31 g with a corresponding root biomass of 2.73±0.90, 2.13±0.42, 1.67±0.70, 1.60±0.82 and 0.90±0.26 g, respectively in the 0, 25, 50, 75 and 100 ml crude oil-contaminated soil (**Table 3.4**).

Shoot and root biomass recorded among the plant species over the 16 weeks growth period provided evidence of high tolerance to the presence of crude oil in soil by these tree species. Although, all tree species seedlings were able to tolerate and grow in the crude oil-contaminated soil, their growth in terms of shoot and root biomass were significantly different rates ($P < 0.05$) (**Appendix 3.34**). Growth was influenced by the concentration of crude oil in the soil, seedlings produced from the oil-treated

soil had lower biomass values as compared with those grown on non-crude oil treated soil. However, seedlings grown on soils with 25 and 50 ml (low and moderate) concentrations produced considerably more shoot and root biomass which compared with non-crude oil-contaminated soils. *D. regia* showed tolerance to the presence of crude oil in soil at various levels of contamination (**Table 3.4**), as it produced more shoot biomass in all the treated soils and the shoots had no significant differences even at 50, 75 and 100 ml oil-treated soil ($P < 0.05$). The mean root biomass of *D. regia* appears most significant ($P < 0.05$) in terms of mean values and tolerance to oil-contamination at all levels, but it shows significant differences ($P < 0.05$). *B. monandra* shoot and root biomass was not significantly different in 0 and 25 ml oil-contaminated soils, but there were significant differences in other treatments. Shoot biomass of *T. tetraptera* showed significant differences for seedlings grown in all the crude oil-contaminated soils, but the seedling root biomass was not significantly different in 0, 25, 50 and 75 ml oil-treated soils, while there was a significant difference in the biomass of seedlings produced in 100 ml oil-treatment ($P < 0.05$) *D. regia* produced the most seedling biomass in both non-crude oil and crude oil-contaminated soils.

Table 3.4: Mean biomass of selected LTS grown in crude oil-contaminated soil 16 WAP* (data represent means ±Standard deviation of 3 replicates)

Treatment (ml)	LTS					
	D. regia		*B. monandra*		*T. tetraptera*	
	Shoot (g)	**Root (g)**	**Shoot (g)**	**Root (g)**	**Shoot (g)**	**Root (g)**
0	107.73±12.91	26.50±1.95	49.00±4.89	12.23±1.98	8.50±0.82	2.73±0.90
25	71.37±3.16	11.27±2.86	49.93±7.13	11.30±0.78	6.70±0.70	2.13±0.42
50	47.80±3.22	7.37±1.06	26.10±9.79	5.50±0.82	5.90±1.15	1.67±0.70
75	41.90±1.55	6.87±1.16	9.87±1.89	2.27±0.42	2.40±1.25	1.60±0.82
100	38.97±8.31	6.50±0.61	5.67±1.22	1.70±0.40	1.13±0.31	0.90±0.26

*Weeks after planting.

3.5. Results of soil analyses

3.5.1. Physicochemical properties of uncontaminated site soil used in the experiment

Selected physicochemical properties of the unpolluted soil used in the experiment are shown in **Table 3.5**. The soil was a slightly acidic (pH 5.9) sandy loam. This pH value is within the optimal soil pH range (4.5-8.2) of surface soils for growth of most plant species in the study area. Soil electrical conductivity was 41.15 μS/cm. The soil organic carbon (SOC) and soil organic matter (SOM) were 2.48 and 4.28%, respectively. SOM is capable of supporting the growth of plant species, as most surface soil (0-15 cm depth) in the study area had SOM in the range 0.1-7.9%. The macronutrients essentially required for normal growth of plant species, such as nitrogen (N), phosphorous (P), potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) were within limits for good plant growth (**Table 3.5**). The site soil had a bulk density of 1.39 g/cm^3 and this may have led to its high good porosity (47.42%). The soil also absorbed a commendable quantity of water (22.6 ml) over a period of 60 minutes. The ability of the soil to absorb water may have influenced the soil moisture content of (10.80%) observed in the site soil. The particle size distribution for sand, silt and clay were 73.20, 20.10 and 6.70%, respectively, thus the soil belongs to the textural class sandy loam. This is the prevalent local soil texture. Thus, the site soil is generally suitable for plant growth.

Table 3.5: Physicochemical properties of uncontaminated site soil used in the experiment

Soil property	Value
pH	5.90
Electrical conductivity	41.15μS/cm
Soil organic carbon (SOC)	2.48%
Soil organic matter (SOM)	4.28%
Nitrogen (N)	2.93 mg/kg
Phosphorous (P)	16.12 mg/kg
Potassium (K)	45.73 mg/kg
Sodium (Na)	40.43 mg/kg
Calcium (Ca)	97.82 mg/kg
Magnesium (Mg)	86.67 mg/kg
Bulk density	1.39 g/cm^3
Soil porosity	47.42 %
Soil capillarity	22.60 ml
Moisture content (by weight)	10.80 %
Particle size distribution:	
Sand (2.0-0.05 mm)	73.20 %
Silt (0.05-0.002 mm)	20.10 %
Clay (<0.002 mm)	6.70 %
Textural class	Sandy loam

*Mg/kg = mg kg^{-1}.

**μS/cm = μS cm^{-1}.

3.5.2. Physicochemical properties of crude oil-contaminated soil planted with leguminous tree species (LTS)

Some physicochemical properties of the experimental soil were measured after contamination with varying amounts of crude oil and subsequently growing selected LTS over 16 weeks (**Table 3.6-3.9**). The mean physicochemical properties of crude oil-contaminated soil planted with LTS at 4 weeks are shown in **Table 3.6**. The effect of the presence of oil in the soil was high at this stage, which negatively affected both plant growth and the soil physicochemical properties. Most of these physicochemical properties were not significantly difference ($P < 0.05$) at 8 and 12 weeks after planting (**Tables 3.7 and 3.8**). However, it was observed that the presence of the LTS on the crude oil-contaminated soil improved soil nutrient conditions at 16 weeks after planting (**Table 3.9**). *D. regia* and *B. monandra* decreased soil acidity and soil planted with *D. regia* increased N and other soil macronutrients, particularly on 0, 25 and 50 ml oil treated soils. The increase in the nutritional status may be due to N-fixation in the root nodules of the LTS and the decay of leaves litter from the plant species onto the soil. Available soil P also increased in the LTS planted soil at the end of the 16-week period.

Table 3.6: Mean physicochemical properties of crude oil-contaminated soils planted with selected leguminous tree species at 4 WAP[*]

LTS	Treatment (ml)	pH	EC[†] (µS/cm)	SOC[††] (%)	SOM[†††] (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
	0	5.92±0.05	77.50±8.03	2.36±0.04	4.08±0.07	2.42±0.03	12.39±0.54	32.72±0.71	30.33±0.46	94.35±0.82	79.05±0.96
D. regia	25	5.84±0.06	65.33±2.96	2.49±0.10	4.31±0.17	1.50±0.26	10.88±0.38	29.14±0.25	27.67±0.94	65.37±0.54	64.52±0.63
	50	5.84±0.05	62.98±3.97	2.41±0.09	4.17±0.17	1.09±0.01	9.10±0.30	26.53±0.34	26.77±0.27	62.25±0.93	60.99±0.13
	75	5.54±0.05	44.57±2.51	2.01±0.18	3.47±0.31	0.70±0.10	8.20±0.05	24.59±0.55	24.70±0.54	59.22±1.02	59.90±0.50
	100	5.30±0.06	39.27±1.08	1.91±0.04	3.31±0.06	0.55±0.04	5.30±0.13	22.75±0.22	23.80±0.70	57.20±0.85	58.79±0.36
B. monandra	0	5.15±0.06	34.05±0.97	2.18±0.07	3.77±0.12	2.16±0.17	11.97±0.51	34.61±1.56	30.62±0.49	83.70±1.73	77.94±0.94
	25	5.40±0.36	25.20±2.27	1.87±0.05	3.23±0.08	0.65±0.06	7.90±0.56	33.68±1.28	26.79±0.81	76.56±0.66	72.33±0.58
	50	5.55±0.13	23.37±1.25	1.77±0.02	3.05±0.03	0.64±0.05	7.29±0.18	27.22±2.32	26.31±0.82	66.23±0.48	60.91±0.54
	75	5.28±0.13	28.77±1.26	1.87±0.03	3.23±0.05	0.60±0.02	6.63±0.41	24.99±0.98	23.04±0.78	66.00±0.17	59.80±0.66
	100	5.51±0.14	23.84±1.03	1.81±0.03	3.12±0.04	0.42±0.03	5.24±0.14	23.86±1.97	21.89±0.70	57.22±0.98	59.23±0.79
T. tetraptera	0	5.82±0.05	25.23±0.75	2.38±0.09	4.11±0.15	2.33±0.06	10.48±0.08	31.30±0.51	29.70±0.43	90.48±0.63	75.37±1.03
	25	5.79±0.03	25.13±0.42	2.21±0.07	3.83±0.12	0.94±0.18	6.80±0.56	30.04±0.98	25.130.34	86.53±0.75	60.51±0.68
	50	5.63±0.07	26.03±0.21	0.70±0.05	1.32±0.08	0.71±0.02	6.28±0.08	25.26±0.54	27.83±0.46	85.37±0.63	59.68±1.24
	75	5.51±0.08	33.50±1.42	0.73±0.01	1.25±0.01	0.67±0.04	6.26±0.48	23.50±0.60	26.72±0.62	66.98±1.16	60.14±0.39
	100	5.57±0.06	30.30±0.98	0.72±0.01	1.25±0.02	0.51±0.03	4.31±0.12	21.81±0.34	25.07±0.93	51.24±1.04	59.09±1.01

Data are mean of 3 replicates ±standard deviation.
[*]Weeks after planting
[†]Electrical conductivity
[††]Soil organic carbon
[†††]Soil organic matter.

Table 3.7: Mean physicochemical properties of crude oil-contaminated soils planted with selected leguminous tree species at 8 WAP[*]

	Treatment (ml)	pH	EC[†] (µS/cm)	SOC[††] (%)	SOM[†††] (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
	0	5.77±0.06	66.10±4.40	2.42±0.05	4.19±0.08	2.63±0.02	12.91±0.04	33.64±0.61	30.44±0.62	89.45±0.95	79.89±0.89
D. regia	25	5.71±0.02	54.33±1.86	2.62±0.03	4.52±0.04	1.61±0.10	12.52±0.08	30.08±0.81	27.85±0.78	65.37±0.73	65.10±0.27
	50	5.70±0.02	56.50±1.87	2.39±0.04	4.12±0.07	0.97±0.02	9.34±0.71	29.04±0.16	26.69±0.33	64.44±0.76	61.91±0.42
	75	5.65±0.04	39.27±0.96	1.97±0.05	3.40±0.08	0.92±0.04	8.80±0.33	26.05±0.43	23.88±0.88	58.59±0.83	60.30±0.11
	100	5.65±0.01	35.83±2.96	1.81±0.03	3.13±0.05	0.66±0.08	6.07±0.18	22.94±0.08	23.45±0.63	57.72±1.27	59.72±0.35
B. monandra	0	5.51±0.03	23.84±1.20	1.81±0.03	3.13±0.05	0.42±0.05	5.24±0.99	23.86±0.51	21.89±0.68	57.22±1.41	59.23±0.85
	25	6.02±0.33	41.93±3.63	1.81±0.03	3.12±0.04	0.76±0.09	8.55±0.35	34.35±1.05	28.00±0.30	80.42±1.15	72.66±0.70
	50	5.95±0.13	44.23±0.71	1.80±0.06	3.12±0.11	0.63±0.04	7.27±0.31	29.01±1.51	25.37±0.74	77.49±1.14	60.20±0.40
	75	6.15±0.31	42.50±1.65	2.23±0.06	3.85±0.10	0.65±0.02	6.53±0.19	26.11±0.26	23.93±0.67	72.00±0.36	57.82±0.33
	100	5.93±0.27	44.93±1.89	1.77±0.01	3.05±0.02	0.49±0.01	6.03±0.50	25.10±0.47	21.82±0.70	59.88±0.46	49.33±0.76
T. tetraptera	0	5.82±0.06	33.17±0.81	2.86±0.07	4.95±0.12	2.24±0.04	11.81±0.14	32.57±0.78	30.97±0.31	91.04±0.72	75.80±0.48
	25	5.74±0.11	29.17±0.83	2.15±0.07	3.73±0.12	0.85±0.15	8.17±0.13	26.64±0.60	26.11±3.49	85.72±0.94	57.75±0.69
	50	5.63±0.01	29.00±0.95	1.89±0.07	3.27±0.12	0.71±0.09	7.07±0.28	24.95±0.71	28.14±0.85	81.58±0.15	60.62±1.07
	75	5.52±0.04	29.70±0.35	2.22±0.18	3.84±0.31	0.66±0.06	6.94±0.33	24.10±0.25	27.70±0.49	66.32±1.03	59.90±0.86
	100	5.80±0.01	28.83±0.49	2.20±0.06	3.80±0.11	0.59±0.01	4.85±0.26	22.99±0.77	25.91±0.72	52.88±0.88	60.17±0.23

Data are mean of 3 replicates ±standard deviation.
[*]Weeks after planting
[†]Electrical conductivity
[††]Soil organic carbon
[†††]Soil organic matter.

Table 3.8: Mean physicochemical properties of crude oil-contaminated soils planted with selected leguminous tree species at 12 WAP[*]

LTS	Treatment (ml)	pH	EC[†] (µS/cm)	SOC[††] (%)	SOM[†††] (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
	0	5.74±0.04	58.67±2.31	2.23±0.01	3.87±0.02	2.69±0.05	12.92±0.04	37.07±0.20	31.37±0.11	89.58±0.94	80.26±0.39
D. regia	25	5.70±0.02	45.93±2.62	2.28±0.07	3.93±0.12	1.68±0.12	12.54±0.07	33.03±1.04	28.99±0.38	66.29±0.53	65.66±0.39
	50	5.67±0.02	43.47±2.17	2.05±0.07	3.55±0.13	1.12±0.08	9.60±0.79	29.93±0.29	26.73±0.31	65.02±0.10	62.22±0.31
	75	5.65±0.05	34.13±2.66	2.04±0.06	3.52±0.11	1.02±0.04	9.02±0.15	27.73±0.48	26.83±0.38	58.87±0.92	60.56±0.40
	100	5.69±0.02	31.03±2.54	1.84±0.02	3.19±0.05	0.87±0.06	6.22±0.10	26.01±0.46	23.66±0.81	57.36±1.27	60.24±0.13
B. monandra	0	5.87±0.05	33.00±1.78	2.23±0.14	3.85±0.24	2.51±0.07	10.49±0.34	36.30±0.50	34.74±0.95	92.21±0.34	79.53±0.58
	25	5.72±0.09	31.63±1.34	2.27±0.13	3.92±0.22	0.73±0.03	9.34±0.29	34.72±1.02	29.28±0.77	82.12±0.94	74.10±1.14
	50	5.87±0.04	33.33±1.36	2.35±0.05	4.07±0.08	0.71±0.02	8.71±0.39	29.32±1.41	27.97±0.32	79.11±0.96	70.11±1.09
	75	5.96±0.15	40.30±1.45	2.20±0.10	3.80±0.18	0.62±0.03	6.99±0.19	26.18±0.26	25.94±0.97	73.68±0.75	58.53±0.76
	100	5.85±0.02	43.03±0.67	2.03±0.08	3.51±0.13	0.52±0.04	6.10±0.16	25.30±0.46	24.25±0.73	62.70±0.61	58.70±0.78
T. tetraptera	0	5.85±0.02	29.33±0.97	2.29±0.06	3.96±0.11	2.32±0.08	11.02±0.51	34.38±0.65	31.67±0.88	93.78±0.86	75.74±0.81
	25	5.86±0.02	28.97±0.67	2.27±0.14	3.93±0.24	0.94±0.08	8.77±0.35	30.19±0.62	28.20±0.42	85.58±0.95	60.03±0.87
	50	5.76±0.09	23.07±0.31	2.20±0.02	3.80±0.04	0.87±0.05	7.98±0.23	28.04±0.19	28.36±0.24	82.47±0.47	59.86±0.26
	75	5.62±0.12	22.40±1.08	1.82±0.09	3.15±0.15	0.81±0.08	7.16±0.39	26.96±0.58	27.72±0.51	66.16±0.90	59.66±0.88
	100	5.72±0.01	24.70±0.85	2.10±0.18	3.63±0.31	0.65±0.07	6.04±0.11	24.25±0.71	26.35±0.22	53.14±0.43	59.52±0.72

Data are mean of 3 replicates ±standard deviation.
[*]Weeks after planting
[†]Electrical conductivity
[††]Soil organic carbon
[†††]Soil organic matter.

Table 3.9: Mean physicochemical properties of crude oil-contaminated soils planted with selected leguminous tree species at 16 WAP*

	Treatment (ml)	pH	EC† (µS/cm)	SOC†† (%)	SOM††† (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
	0	5.76±0.22	45.40±2.81	2.68±0.06	4.64±0.11	2.41±0.05	13.28±0.26	39.22±1.14	32.36±0.50	91.11±1.53	82.39±0.44
D. regia	25	5.69±0.05	38.67±1.32	2.58±0.01	4.45±0.02	1.86±0.06	12.57±0.09	35.58±0.73	30.20±0.20	66.62±0.63	68.26±0.29
	50	5.70±0.01	33.77±2.51	2.56±0.07	4.41±0.12	1.24±0.15	10.82±0.12	29.96±0.28	27.30±0.39	65.77±0.29	62.31±0.18
	75	5.70±0.01	23.37±0.98	2.51±0.02	4.33±0.05	1.08±0.03	10.02±0.21	25.37±0.68	27.24±0.30	62.53±0.52	61.33±0.47
	100	5.48±0.03	20.80±2.07	2.17±0.09	3.75±0.17	0.95±0.09	7.27±0.15	26.04±0.48	24.06±0.51	57.84±0.89	60.29±0.56
B. monandra	0	6.03±0.23	37.87±0.95	2.49±0.07	4.31±0.13	2.48±0.03	10.61±0.22	43.96±1.20	37.24±0.75	92.79±0.26	79.10±0.92
	25	5.98±0.11	32.07±0.76	2.12±0.16	3.67±0.27	0.97±0.01	10.00±0.45	40.52±0.37	30.99±0.79	83.54±0.81	77.49±0.99
	50	5.95±0.39	33.47±1.18	2.35±0.02	4.07±0.05	0.85±0.05	8.99±0.30	36.35±0.55	29.35±0.66	80.25±0.82	73.61±0.59
	75	6.03±0.17	35.13±0.50	2.04±0.09	3.52±0.14	0.71±0.06	7.64±0.41	33.43±1.26	26.83±0.21	73.85±0.60	61.10±0.90
	100	5.89±0.21	39.30±0.75	2.17±0.09	3.75±0.17	0.61±0.02	6.18±0.03	29.39±1.07	24.38±0.60	65.58±0.80	59.37±0.89
T. tetraptera	0	5.84±0.04	32.63±0.97	2.37±0.05	4.09±0.08	2.45±0.07	10.53±0.21	35.82±0.70	33.31±0.66	94.52±0.63	76.88±0.66
	25	5.79±0.16	33.17±0.70	2.38±0.07	4.12±0.12	0.88±0.07	10.13±0.34	31.28±0.45	29.53±0.49	85.23±0.37	61.29±0.33
	50	5.83±0.02	32.10±0.56	2.52±0.13	4.35±0.23	0.77±0.11	9.95±0.64	28.17±0.25	28.61±0.18	82.57±0.48	60.96±0.25
	75	5.58±0.02	24.97±0.72	1.96±0.11	3.40±0.18	0.73±0.06	7.59±0.42	27.05±0.13	28.03±0.21	65.79±0.43	60.78±0.50
	100	5.60±0.02	22.97±0.96	1.80±0.03	3.11±0.05	0.66±0.07	6.24±0.23	24.90±0.24	26.82±0.30	54.16±0.26	60.52±0.48

Data are mean of 3 replicates ±standard deviation.
*Weeks after planting
†Electrical conductivity
††Soil organic carbon
†††Soil organic matter.

3.5.3. Microbial count in the crude oil-polluted soil planted with LTS

Soil micro-organisms play a vital role in plant growth in terms of nutrient cycling and replenishment in the soil. These microbes are also important for hydrocarbon degradation in soil. Their role includes the degradation and biotransformation of complex petroleum compounds into simple harmless compounds (Oluyege *et al*., 2011). Microbial counts in the crude oil-contaminated soil planted with LTS were used to determine microbial population, especially heterotrophic bacteria and fungi counts. Microbial populations in the rhizosphere of *D. regia*, *B. monandra* and *T. tetraptera* in the oil-contaminated and non-oil contaminated soils were obtained for each plant species at the end of the 16 weeks growth period and compared with the microbial population of the site soil. A total of 8.33×10^5 heterotrophic bacteria were observed in the site soil and 3.93×10^5, 8.67×10^4, 7.67×10^4 and 6.67×10^4 were observed in 25, 50, 75 and 100 ml crude oil-contaminated soil, respectively, at 24 hours after contamination. Similarly, a total of 2.37×10^3, 2.50×10^3, 2.43×10^3, 2.17×10^3 and 1.03×10^3 heterotrophic fungi were recorded in the 0, 25, 50, 75 and 100 ml crude oil-contaminated soils, respectively, 24 hours after contamination (**Table 3.10**). There were more bacteria and fungi counts in the site soil than in the oil-treated soils and the microbial population decreased with increased oil concentration significantly. The results indicate that oil contamination/spillage alters the population of indigenous soil microbes, particularly at high concentrations.

Table 3.10: Total number of heterotrophic bacteria and fungi in the rhizosphere of non-contaminated and contaminated soil without LTS

LoC* (ml)	Heterotrophic bacteria count (cfu/g)	Heterotrophic fungi count (cfu/g)
0**	8.33×10^5	2.37×10^3
25	3.93×10^5	2.50×10^3
50	8.67×10^4	2.43×10^3
75	7.67×10^4	2.17×10^3
100	6.67×10^4	1.03×10^3

*Level of contamination
**Site soil.

The introduction of LTS seedlings into the crude oil-contaminated soil significantly increased the microbial population of the contaminated soil at 16 WAP. Crude oil-contaminated soil planted with *D. regia* had heterotrophic bacteria counts of 4.33 x 10^5, 8.27 x 10^5, 5.30 x 10^5, 3.60 x 10^5 and 2.53 x 10^5 cfu/g in 0, 25, 50, 75 and 100 ml oil-treated soil, respectively (**Table 3.11**). Total heterotrophic bacteria counts of 2.97 x 10^5, 6.33 x 10^5, 6.33 x 10^5, 3.43 x 10^5 and 2.33 x 10^5 cfu/g were observed in the 0, 25, 50, 75 and 100 ml oil-treated soil planted with *B. monandra*. Contaminated soil planted with *T. tetraptera* recorded bacterial counts of 2.27 x 10^5, 3.83 x 10^5, 4.23 x 10^5, 2.30 x 10^5 and 1.93 x 10^5 cfu/g in 0, 25, 50, 75 and 100 ml oil-treated soil. Fungi counts in soil planted with *D. regia* were 3.20 x 10^3, 4.73 x 10^3, 4.03 x 10^3, 3.13 x 10^3 and 2.40 x 10^3 cfu/g. A total of 2.47 x 10^3, 3.10 x 10^3, 2.53 x 10^3, 2.03 x 10^3 and 1.27 x 10^3 cfu/g heterotrophic fungi were observed in the soil planted with *B. monandra,* while 2.20 x 10^3, 3.00 x 10^3, 2.70 x 10^3, 2.10 x 10^3 and 1.20 x 10^3 cfu/g heterotrophic fungi were measured in 0, 25, 50, 75 and 100 ml, respectively, crude oil-contaminated soil planted with *T. tetraptera* (**Table 3.11**).

Table 3.11: Total number of heterotrophic bacteria and fungi (cfu/g) in the rhizosphere of non-contaminated and contaminated soil planted with LTS

LoC* (ml)	*D. rgia*		*B. monandra*		*T. tetraptera*	
	HBC (x 10^5)†	HFC (x 10^3)††	HBC (x 10^5)†	HFC (x 10^3)††	HBC (x 10^5)†	HFC(x10^3)††
0**	4.33	3.20	2.97	2.47	2.27	2.20
25	8.27	4.73	6.33	3.10	3.83	3.00
50	5.30	4.03	6.33	2.53	4.23	2.70
75	3.60	3.13	3.43	2.03	2.30	2.10
100	2.53	2.40	2.33	1.27	1.93	1.20

*Level of contamination
**Site soil
†Heterotrophic Bacteria Count.
††Heterotrophic Fungi Count.

In all the plant-treated oil-contaminated soils, total soil microbial counts increased over time in low and moderately crude oil-contaminated soil, than in the high crude oil-contaminated soil. Presence of oil in soil enhances microbial growth, particularly at low concentrations. The increase in the microbial population in 25 and 50 ml crude oil-contaminated soil may have been enhanced by the oil and some of the microbes are unable to withstand high oil concentrations soil samples contaminated with 75 and 100 ml and died. However, those that die can return nutrients to the soil for an enhanced nutrient status for plant growth.

Heterotrophic bacteria and fungi are capable of utilizing hydrocarbons and thereby degrade the complex hydrocarbon chains to simpler ones. Microscopic examination (**Plate 2.19**) of stained slides prepared from the oil-treated soil revealed predominantly bacteria and fungi isolates and identification of these isolates were determined using physiological and morphological features of the isolates and compared with taxonomic standards (**Figure 3.14**). Bacteria isolates in the oil-treated soils were *Pseudomunas* spp., *Bacillus* spp., *Nocardia* spp., *Micrococcus* spp., *Achromobacter* spp. and *Arthrobacter* spp. The fungi isolates were *Aspergillus* spp., *Fusarium* spp., *Saccharomyces*, *Mucor* spp. and *Rhodotorula* spp. and *Rhizopus* spp. All these are good hydrocarbon utilizing soil micro-organisms capable of degrading hydrocarbon pollutants in the soil (Chikere *et al*. 2011; Oluyege *et al*. 2011; Omare and Agwu, 2012).

One-way ANOVA (**Appendix 3.35)** showed that both soil bacterial and fungal growth were statistically significantly different ($P < 0.05$) in soil planted with *D. regia*. Bacterial populations in *B. monandra* were also significantly different in the treatments, but fungal counts were not significantly different ($P > 0.05$). Similarly, both bacterial and fungal counts were not significantly different in soil grown with *T.*

tetraptera. Soil planted with *D. regia* tended to have the highest microbial populations capable of degrading oil hydrocarbons.

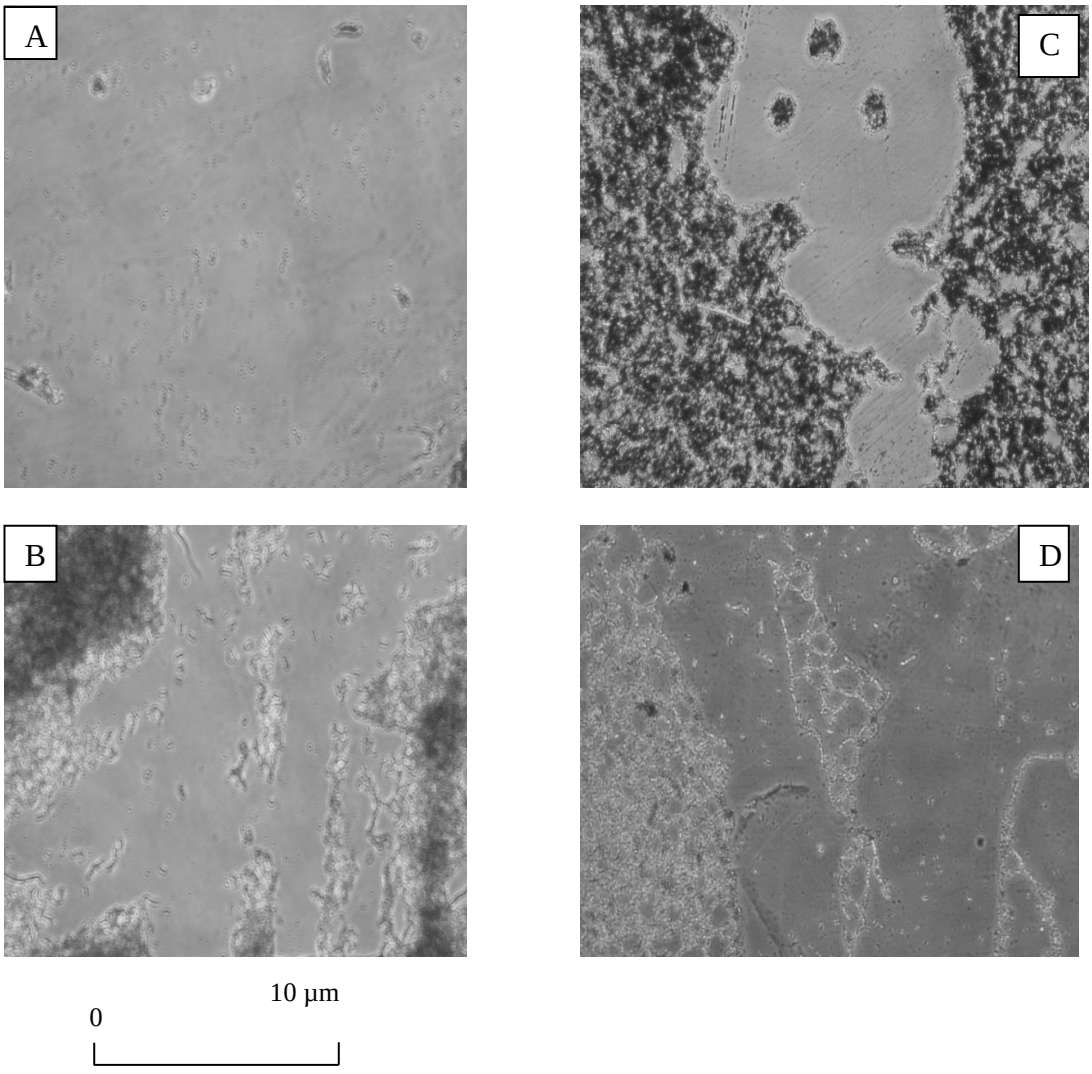


Figure 3.14: Selected photographs of microbial colonies found in crude oil-contaminated soil on agar plates: (A) *Micrococcus* spp (B) *Achromobacter* spp (C) *Fusarium* spp (D) *Mucor* spp.

3.5.4. Hydrocarbon degradation and removal from the rhizosphere of leguminous tree species and non-planted crude oil-contaminated soil

The Gas Chromatography Mass Spectrophotometer (GC-MS) was calibrated using the 10 ppm Total Petroleum Hydrocarbon (TPH) prepared standard for hydrocarbon determination. The prepared standard is capable of aiding the GC-MS to detect both polyaromatic and aliphatic hydrocarbons present in both the crude oil and crude oil-

contaminated soil. The aliphatic hydrocarbon components (**Appendix 3.36**) and polyaromatic hydrocarbons (**Appendix 3.37**) detectable by the TPH standards are listed. Chromatographic peaks of these TPH (aromatic and aliphatic) compounds observed after runs on GC-MS are presented in **Figure 3.15** and the standard was used to calibrate the GC-MS to determine TPH present in the crude oil-contaminated soil planted with LTS and the non-planted soil. The hydrocarbon compounds and their peaks present in a typical Nigerian light crude oil used in this experiment are presented in (**Figure 3.16**). The physicochemical properties of a typical Nigerian light crude oil (**Table 3.12**) show that the crude oil is less viscous and therefore, has potential to mix thoroughly with the soil and this is the situation of soil in the crude oil-bearing regions of Nigeria during oil spillages.

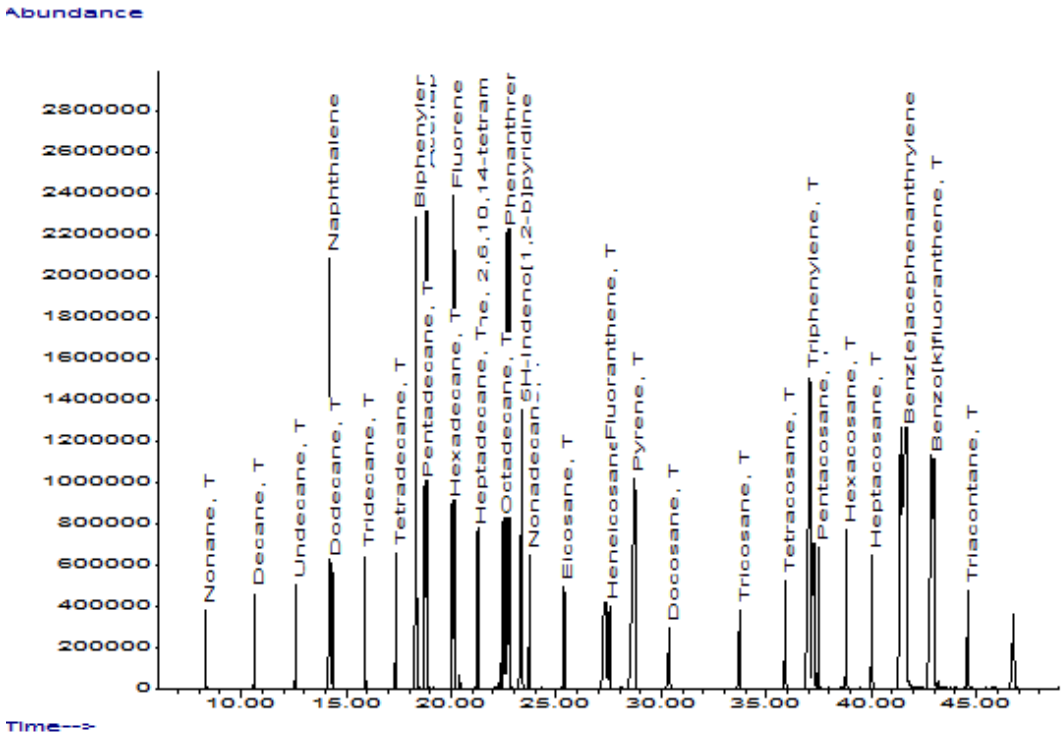


Figure 3.15: Chromatograph of TPH standard formation showing detectable hydrocarbon compounds.

Table 3.12: Physicochemical properties of typical Nigerian light crude oil (Source: Osuji *et al*., 2005).

Parameter	Value
Sulphur	0.14%
Specific gravity	0.8398
API gravity 15.5ºC	37
Viscosity (cSt) at 25ºC	4.09
Wax content	3.8%
Pour-point (-18ºC)	23
Surface tension (/Nm)	0.02041
Refractive index	1.472

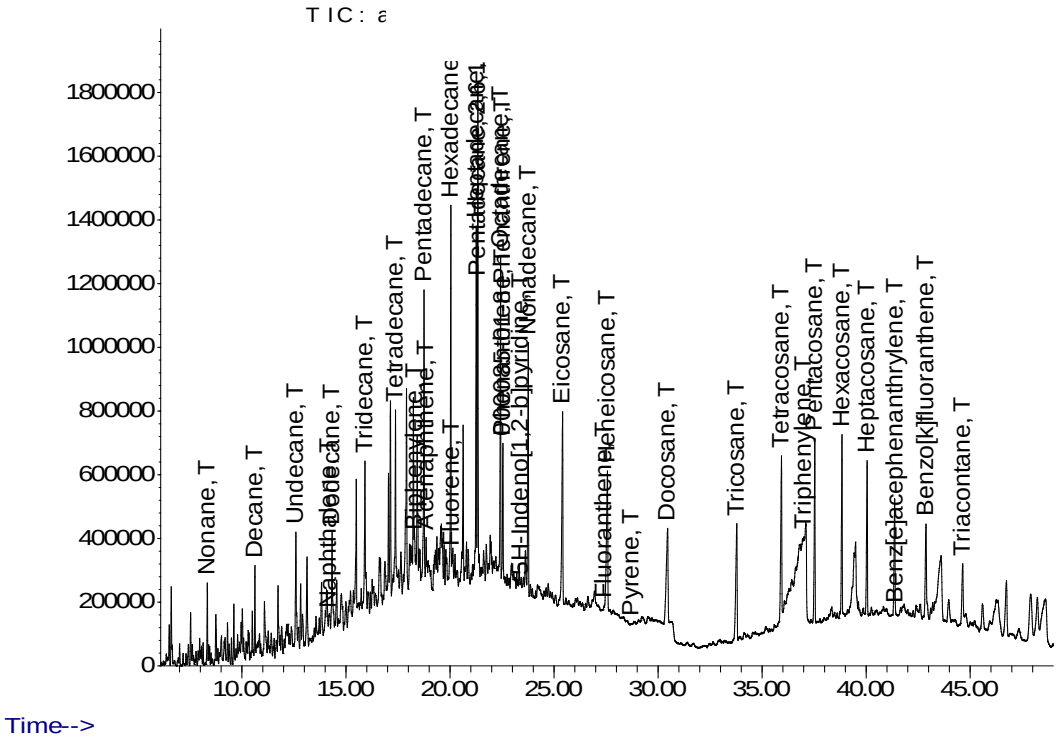
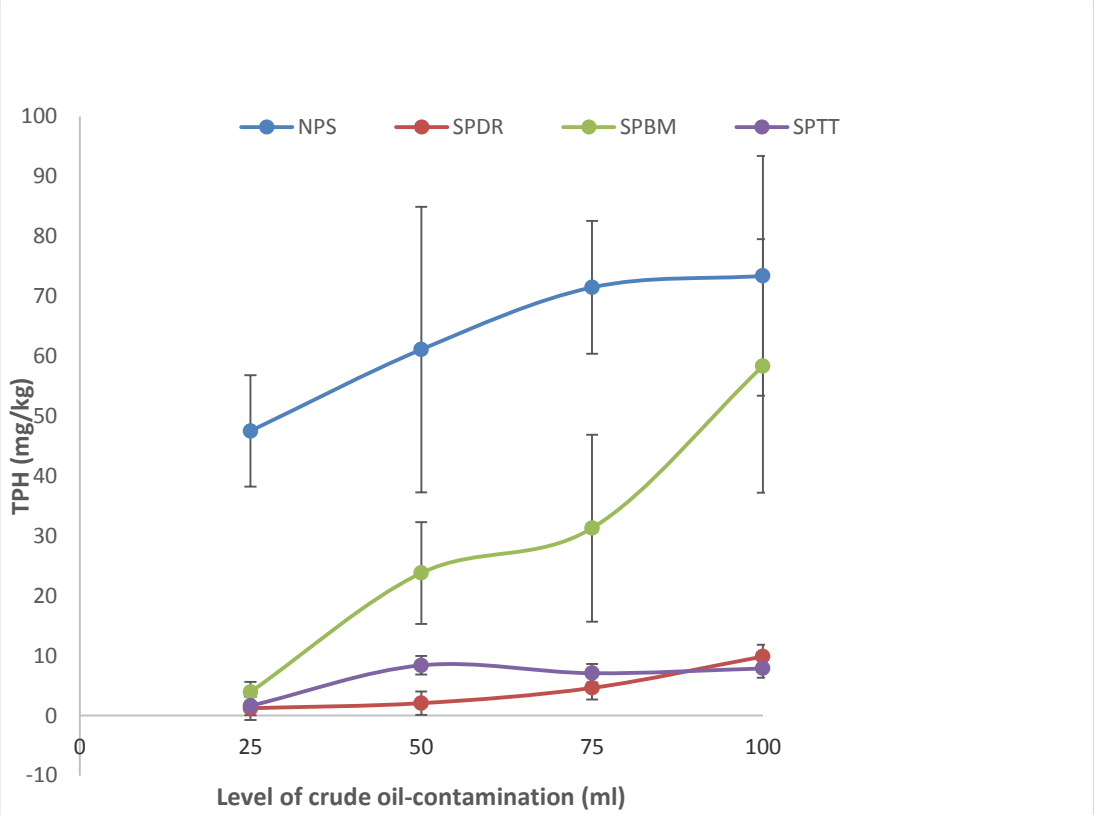


Figure 3.16: Chromatograph of hydrocarbon compound constituents of a typical Nigerian light crude oil.

Assessment of TPH concentration over 16 weeks shows the influence of LTS rhizospheric activity on crude oil compound removal from oil-contaminated soil. GC-MS showed TPH available in crude oil as 190.06±0.00 mg/kg. The available TPH in the various crude oil contamination levels in the LTS rhizosphere and non-planted soil at the end of 16 weeks showed that crude oil compounds were more degraded in soils planted with LTS than non-LTS planted soil (**Figure 3.17**, **Table 3.13**). The amount of TPH in LTS planted soil was lower than non-planted soil at the end of the experiment. Similarly, the TPH available in crude oil-contaminated soil planted with *D. regia* and *T. tetraptera* were significantly ($P < 0.05$) less than in crude oil-contaminated soil planted with *B. monandra*. Microbial populations in the crude oil-contaminated soil can influence hydrocarbon degradation in the crude oil-contaminated soil. These microbes can convert complex hydrocarbons to simple ones and breakdown hydrocarbons to liberate carbondioxide (CO_2) and oxygen (O_2) for their respiration.



NPS = Non-planted crude oil-contaminated soil.
SPDR = Crude oil-contaminated soil planted with *D. regia*.
SPBM = Crude oil-contaminated soil planted with *B. monandra*.
SPTT = Crude oil-contaminated soil planted with *T. tetraptera*.
Error bars = ±Standard deviation.

Figure 3.17: TPH in the crude oil-contaminated rhizosphere of LTS and non-planted soil at 16 WAP.

In non-planted soil, mean TPH was 47.52±9.30, 61.11±23.82, 71.48±11.09 and 73.40±20.0 mg/kg in 25, 50 75 and 100 ml, respectively, in crude oil-contaminated soil at 16 WAP. There were no significant differences in TPH removal in all non-planted treatments. *D. regia* planted soil had a mean TPH of 1.24±0.46, 2.08±1.35, 4.36±1.70 and 9.87±2.48 mg/kg in 25, 50, 75 and 100 m, respectively, at 16 WAP (**Table 3.13**). TPH in the *D. regia* planted soils were not significantly different in 25, 50 and 75 ml crude oil-contaminated soil, but was significantly different in 100 ml treatment when compared with control. However, a significant decrease in TPH was observed in all treatments when compared with non-planted soil (**Appendix 3.38**).

TPH degradation in the soil planted with *D. regia* had more degraded hydrocarbon compounds. There were significant differences in the TPH available in all treatment soils planted with *B. monandra* at 16 WAP, but it was not significantly different from the non-planted soil. Similarly, in T. *tetraptera* planted soil, there were significant differences between 25 ml and other treatments, but there were no significant difference between 50, 75 and 100 ml crude oil-contaminated soil (**Table 3.13**). However, TPH degradation in *T. tetraptera* treated soil was not significantly different (**Appendix 3.38**). TPH degradation was not significantly different in *B. monandra* and *T. tetraptera*, but was significantly different in *D. regia* when compared with non-planted soil 16 WAP (**Appendix 3.38**). Selected chromatographs of TPH in crude oil-contaminated soils are presented in **Figure 3.18.** Aliphatic hydrocarbon C_9-C_{30} were notably degraded, while Naphthalene, Biphenylene, Fluorene, Phenathrene and 5H-Indeno[1,2-b]pyridine were the main polyaromatic hydrocarbons degraded in the crude oil-contaminated soils. The rate of TPH degradation was high in the low and moderately oil-treated soils, which could be attributed to the ability of microbes adapt and survive in such conditions. The breeding and respiratory activities of these microbes may influence the significant TPH degradation witnessed in the low and moderately crude oil-contaminated soils.

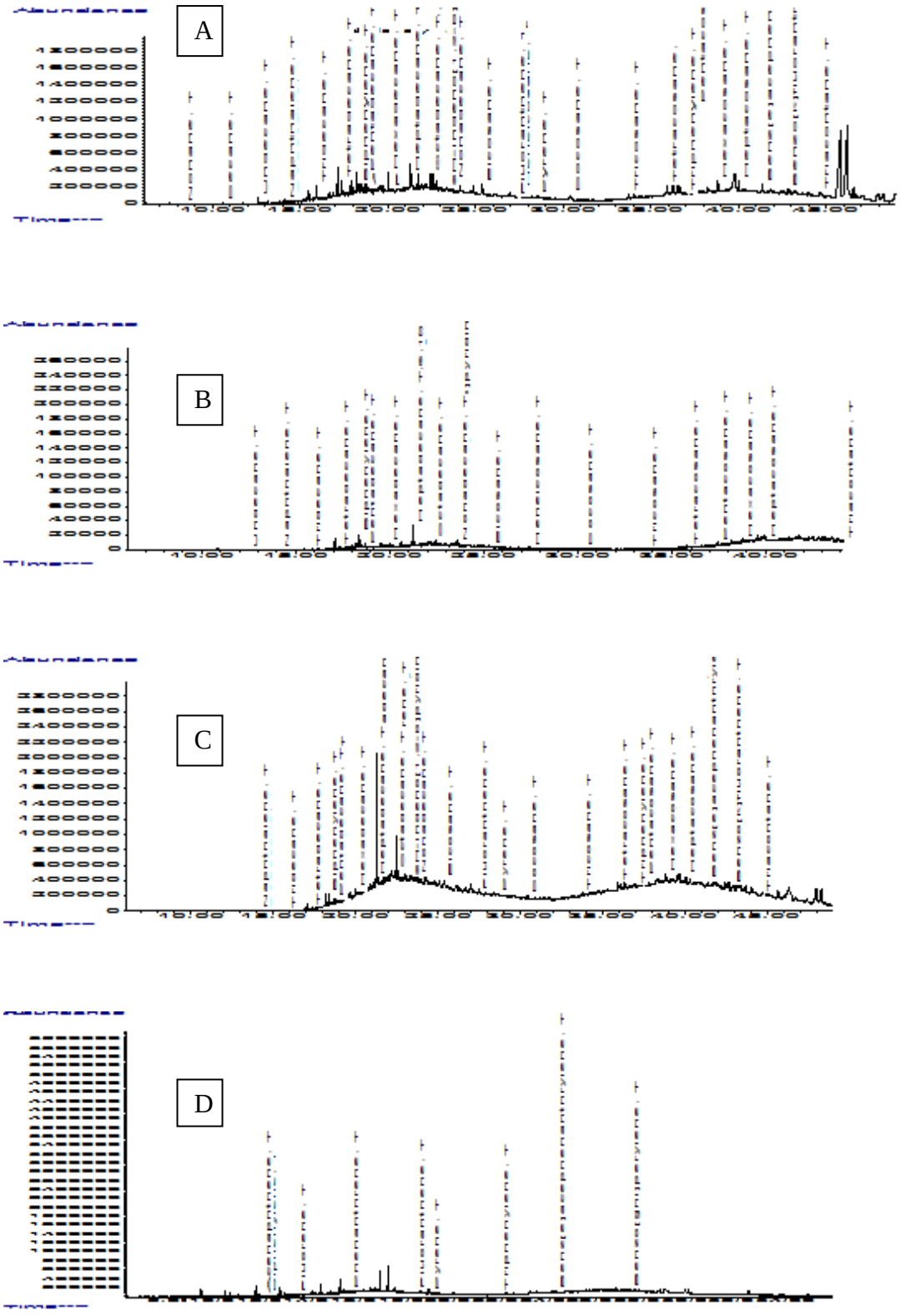


Figure 3.18: Selected chromatographs of TPH in crude oil-contaminated soil: (A) Non-planted 25 ml crude oil-contaminated soil (B) 50 ml crude oil-contaminated soil planted with *D. regia* (C) 100 ml crude oil-contaminated soil planted with *B. monandra* (D) 75 ml crude oil-contaminated soil planted with *T. tetraptera*.

Table 3.13: Mean (±Standard deviation, n = 3) TPH in crude oil-contaminated soil remediated with LTS at 16 WAP[#]

Treatment (ml)/LTS	TPH (mg/kg) in non-planted and LTS planted rhizosphere			
	Non-planted soil	***D. regia***	***B. monandra***	***T. tetraptera***
25	47.52±9.30 (142.54)	1.24±0.46 (188.82)	3.95±1.70 (186.11)	1.65±1.30 (188.41)
50	61.11±23.82 (128.95)	2.08±1.35 (187.98)	23.81±8.49 (166.25)	8.40±2.46 (181.66)
75	71.48±±11.09 (118.58)	4.63±1.70 (185.43)	31.30±15.61 (158.76)	7.08±3.31 (182.98)
100	73.40±20.01 (116.66)	9.87±2.48 (180.19)	58.36±21.17 (131.70)	7.88±3.62 (182.18)

[#]Weeks After Planting (WAP).

TPH degradation over a period of 16 WAP in parenthesis.

Some of the TPH in the crude oil-contaminated soil were transferred to the roots and shoots of the tree species, perhaps during nutrient uptake. Some 3.92-7.99 mg/kg TPH were detected in *D.* regia, ~2.53-4.55 mg/kg TPH in *B. monandra* and 0.80-2.91 mg/kg TPH in *T. tetraptera* after their seedlings were grown in the crude oil-contaminated soil for 16 weeks. These results indicate plant species, when grown on contaminated soil, have the potential to take up some of the hydrocarbon contaminants through the process of diffusion based on their octnol water partition co-efficient. Aliphatic hydrocarbon compounds detected in the plant system are aliphatic hydrocarbons, C_9-C_{30}, but no aromatic hydrocarbons were detected. Some of the aliphatic hydrocarbons (such as octane, nonane and decane) present in roots and shoots are volatile organic compounds that can be phytovolatilized. These results therefore, indicate that phytodegradation and/or potentially phytovolatilization took place in the plants system. Detection of these compounds in the plants' system may be responsible for some eco-physiological problems in plants, such as growth retardation, especially at high amounts of oil. Selected chromatographic peaks of the TPH in plant tissues are presented in **Figure 3.19**.

3.5.5. Establishment of LTS in the field (Forest of Ayodele)

The growth and development of the selected LTS were assessed under field conditions after the initial early growth period of 16 weeks in the greenhouse. All the LTS were able to grow in the field. However, their growth rate varied (**Table 3.14**) although they were all grown under the same climatic and other environmental conditions. These growth parameters are often used to determine growth success rate of plant and growth

media. *D. regia* had a mean height of 143.66±16.51, 177.62±13.68 and 205.32±13.04 cm at 24, 48 and 72 WAP, respectively. Corresponding girth values were 2.78±0.78 and 9.20±1.10 cm over the same period. *B. monandra* had a slightly lower height of 97.64±9.08, 115.64±12.98 and 132.62±11.36 cm than D. *regia* at the various growth periods. Mean seedling girth in *B. monandra* was 1.14±0.22, 1.78±0.52 and 3.34±0.55 cm at 24, 48 and 72 WAP. Mean numbers of leaves in *B. monandra* were 19, 22 and 25, while the mean number of young branches produced were 2, 5 and 8 at 24, 48 and 72 WAP in the field.

Table 3.14: Growth of selected leguminous tree species (LTS) at intervals in field conditions after the initial early growth period of 16 weeks under greenhouse conditions

Period/LTS	*D. regia*	*B. monandra*	*T. tetraptera*
	Mean plant height (cm), n = 5		
24 WAP	143.66±16.51	97.64±9.08	54.76±4.67
48 WAP	177.62±13.68	115.64±12.98	70.14±8.63
72 WAP	205.32±13.04	132.62±11.36	90.28±8.37
	Mean plant girth (cm), n = 5		
24 WAP	2.78±0.78	1.14±0.22	0.55±0.05
48 WAP	4.02±0.20	1.78±0.52	0.65±0.08
72 WAP	9.20±1.10	3.34±0.55	0.91±0.12
	Mean leaf production, n = 5		
24 WAP	40.00±10.00	19.00±7.00	11.00±2.00
48 WAP	50.00±9.00	22.00±8.00	14.00±3.00
72 WAP	64.00±4.00	25.00±9.00	17.00±3.00
	Mean branch production, n = 5		
24 WAP	5.00±2.00	2.00±1.00	0.00±0.00
48 WAP	9.00±1.00	5.00±1.00	0.00±0.00
72 WAP	11.00±2.00	8.00±2.00	2.00±1.00

T. tetraptera had a mean height of 54.76±4.67, 70.14±8.63 and 90.28±8.37 at 24, 48 and 72 WAP and the mean seedling girth during the study period was <1.00 cm. *T. tetraptera* seedlings grown in the field produced between 10 and 17 leaves and branch production was noticed in the species at 72 WAP. *D. regia* recorded strong growth in terms of height, girth leaves and branch production over the other two species in the field.

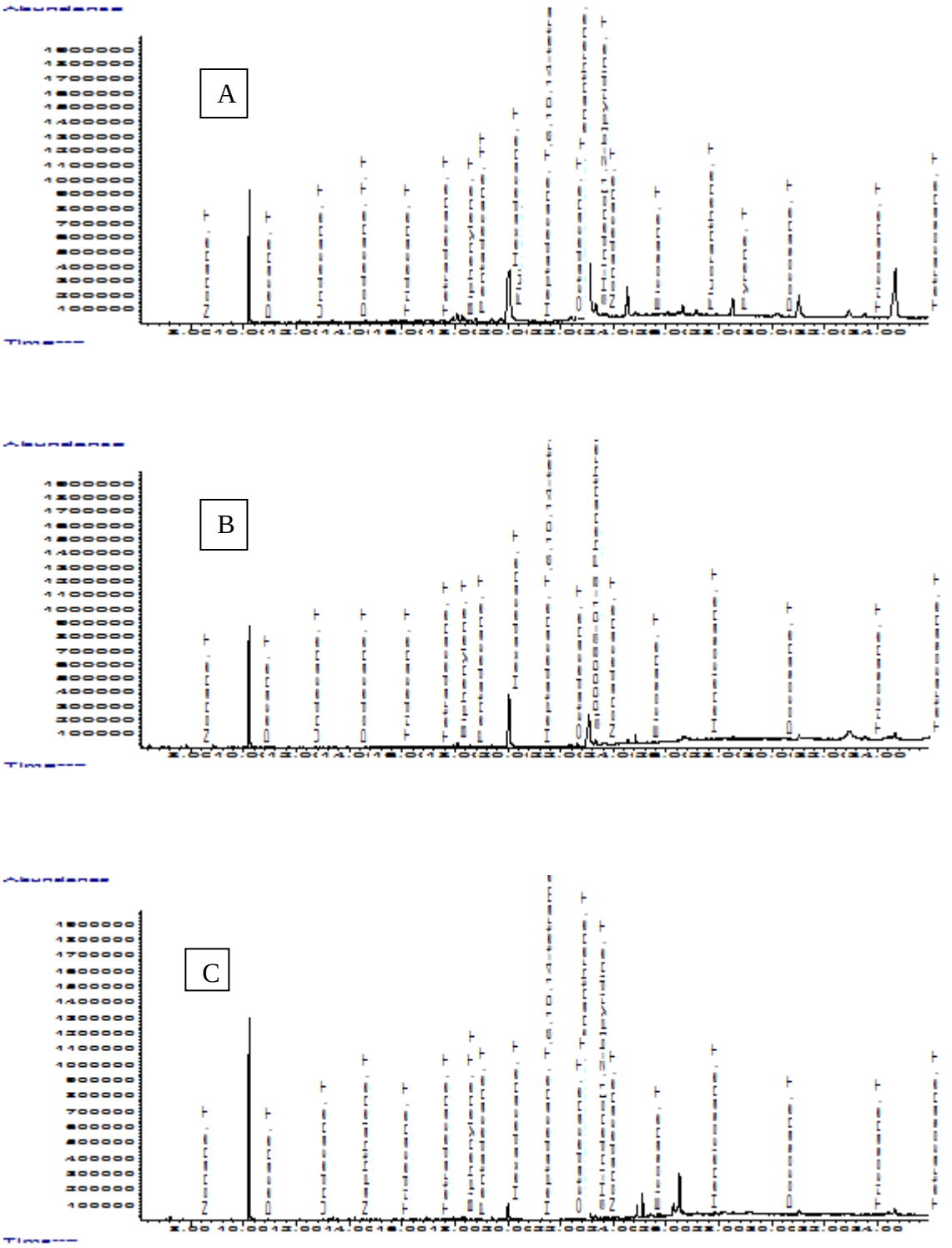


Figure 3.19: Selected chromatographs of TPH in leguminous tree species grown on crude oil-contaminated soil: (A) *D. regia* grown on 25 ml crude oil-contaminated soil (B) *B. monandra* 50 ml crude oil-contaminated soil (C) *T. tetraptera* grown on 25 ml crude oil-contaminated soil.

3.6. Conclusions

The success of phytoremediation work depends partly on the ability of plants to germinate and establish cover on the oil-contaminated site. The selected plant species should exhibit tolerance in such soil and be able to produce adequate root-soil relationships to produce the desired oil degradation in the oil-contaminated soil. Selection of plant species for phytoremediation is a critical step, which involves evaluation of germination potentials followed by a greenhouse study can help identify plant species that can tolerate, survive and thrive through the initial establishment period (Bamidele and Agbogidi, 2006; Kulakow *et al*., 2000). *D. regia* out *B. monandra* and *T. tetraptera* in terms of germination, early growth response in the greenhouse, soil physicochemical properties improvement, microbial densities (which correspond to strong hydrocarbon degradation and establishment in the field). This species had significantly better growth performance in the oil-contaminated soil conditions and will have a good chance of producing initial vegetation establishment that will enhance long-term ecosystem processes and oil degradation in oil-contaminated soil.

CHAPTER FOUR

Results of Plant Experiment II

4.0. Introduction

This chapter reports experimental data and statistical analyses for the three Leguminous Tree Species (LTS) (*Albizia adianthifolia*, *Albizia odoratissima* and *Pterophorum pterocarpum*) investigated in 2014. The report covers germination of the selected tree species, early growth performance in the Greenhouse, plant biomass, nodulation, soil physicochemical and microbial properties, hydrocarbon degradation and establishment of LTS seedlings of the species in the field.

4.1. Percentage germination of LTS in crude oil-contaminated soil water extracts

The percentage seed germination and COV of germination of the selected LTS moistened with varying crude oil-contaminated soil water extracts is presented (**Table 4.1**). The ability of seeds of the selected LTS to germinate varied greatly with respect to oil treatment (**Figure 4.1**). Seed germination and COV of germination of the LTS seeds studied was strongly influenced by the amount of oil in treated soils. Low oil concentration in the extract results in high rates of seed germination and COV of germination of the LTS. *P. pterocarpum* had seed percentage germination rates of 96, 90, 76, 76 and 64%, with high COV, when moistened with 0, 25, 50, 75 and 100 ml crude oil-contaminated soil water extract, respectively. Seed germination rate of *A. odoratissima* was significantly lower than that of *P. pterocarpum*. *A. odoratissima* had a percentage seed germination of 90, 80, 76, 56 and 44% when moistened with 0, 25, 50,

75 and 100 ml crude oil-contaminated soil water extract, respectively. Similarly, seed germination rates of 84, 72, 62, 56 and 42% occurred in *A. adianthifolia* seeds grown in 0, 25, 50, 75 and 100 ml crude oil-contaminated soil water extract, respectively. Seed germination rates also decreased significantly along the concentration gradient in the oil-treated soil water extract used to moisten seeds. In all the seeds investigated, it was noticeable that both seed germination and COV of germination correlated along the oil-concentration gradient in the oil-treated soil extracts.

Table 4.1: Percentage seed germination and co-efficient of velocity of germination of selected LTS in crude oil-contaminated water extracts

Treatment (ml)	***P. pterocarpum***		***A. odoratissima***		***A. adianthifolia***	
	Gt* (%)	**CoV** (%)**	**Gt* (%)**	**CoV** (%)**	**Gt* (%)**	**CoV** (%)**
0	96	68.71	90	68.00	84	65.16
25	90	64.09	80	60.25	72	58.91
50	76	62.90	76	60.23	62	56.73
75	76	62.82	56	59.53	56	56.18
100	64	60.66	44	53.21	42	45.35

*Germination (%)

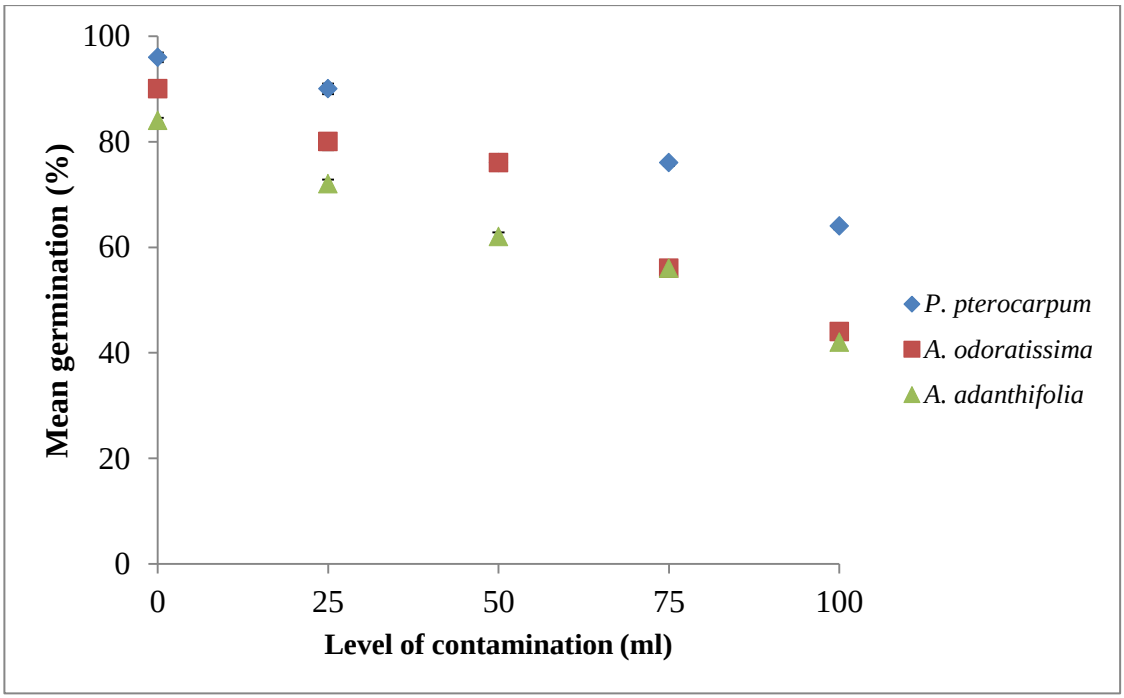
**Co-efficient of Velocity (%).

Statistical analysis by one-way ANOVA ($P < 0.05$) shows significant differences among the LTS investigated (**Appendix 4.1a**). Evaluation of individual tree species mean

germination response in the contaminated soil by one-way ANOVA ($P < 0.05$) shows that there were no significant differences for *P. pterocarpum* seeds germination moistened with 0 and 25 ml crude oil-contaminated soil water extracts. However, there were significant differences in the germination of LTS seeds when 50, 75 and 100 ml oil-treated soil extracts were used. *P. pterocarpum* seed germination and contamination were strongly correlated ($R^2 = -0.841$; $n = 5$; $P < 0.05$) (**Appendix 4.2**). *P. pterocarpum* seeds germinated better than other LTS during the germination experiment. There were significant differences in seed germination of *A. odoratissima* and *A. adianthifolia* at ($P < 0.05$) by one-way ANOVA in the various extracts. The rate of seed germination in the contaminated extracts also strongly correlates for *A. odoratissima* and *A. adianthifolia* ($R^2 = -0.875$; $n = 5$; $P < 0.05$) and ($R^2 = -0.888$; $n = 5$; $P < 0.05$), respectively (**Appendix 4.2**).

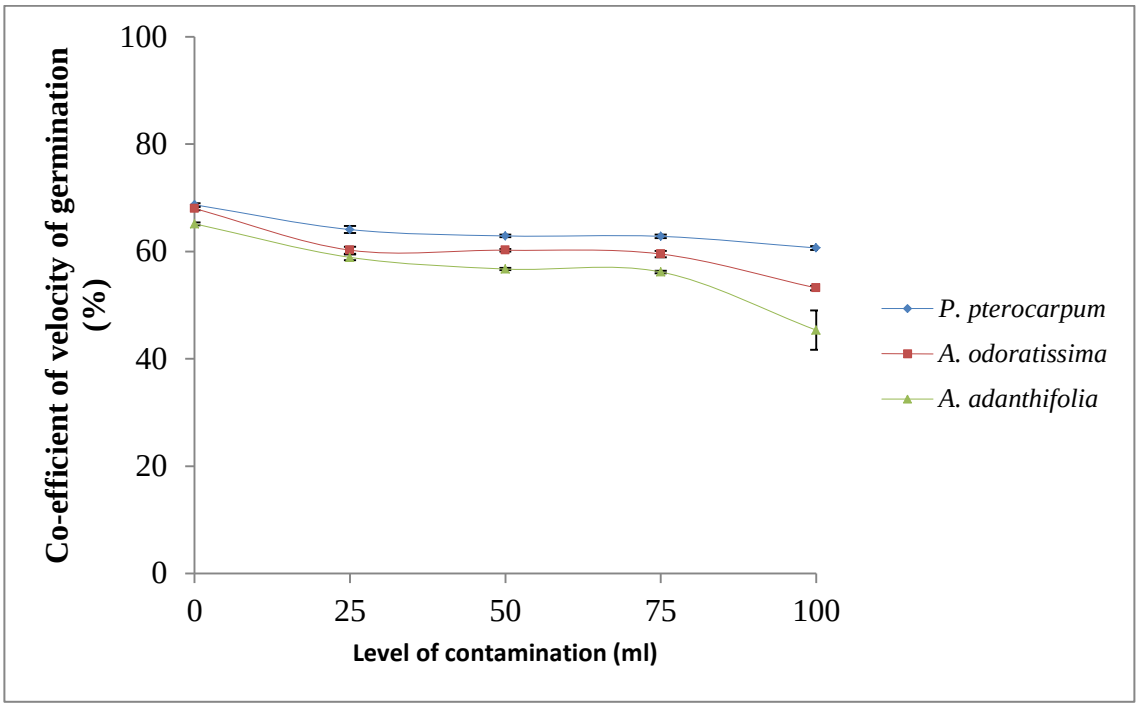
The level of contamination in the LTS also influenced the COV of germination (**Table 4.1, Figure 4.2**), and the speed of germination was significantly different at ($P < 0.05$) in *P. pterocarpum* and *A. odoratissima* seeds, but was not significantly different in *A. adianthifolia* seed as compared with the control by one-way ANOVA (**Appendix 4b**). Similarly, mean of COV of germination was significantly different ($P < 0.05$) in *P. pterocarpum* and *A. odoratissima* in all treatments. The mean was not significantly different in *A. adianthifolia* moistened with 0, 25, 50 and 75 ml crude oil water extracts, but there were significant difference between 100 ml and other treatments at ($P < 0.05$). Both the germination percentage and COV of germination were influenced by the amount of oil in soil and, therefore, germination was concentration dependent. The

complimentary results obtained between percentage mean germination and COV of germination may prove that the percentage mean germination is strongly influenced by the concentration of oil in soil (**Table 4.2**). All the LTS germinated at different rates when moistened with crude oil contaminated water extracts, but germination was concentration dependent. Percentage mean germination decreased with increased oil concentrations in the extracts. Although, the germination percentage of the selected tree species varied, all LTS tolerated contamination and germinated (**Table 4.1**). Similar observations were made among the tree species earlier investigated and reported in **Section 3.1**. These tested tree species particularly, *P. pterocarpum*, therefore tends to hold promise for phytoremediation of crude oil contaminated soil and re-vegetation of such soils, even at high oil concentrations.



Error bars = ±Standard deviation.

Figure 4.1: Comparison of percentage mean germination among selected LTS moistened with varying concentration of crude oil-contaminated water extracts.



Error bars = ±Standard deviation.

Figure 4.2: Comparison of Co-efficient of Velocity of germination among selected LTS moistened with varying concentration of crude oil-contaminated water extracts.

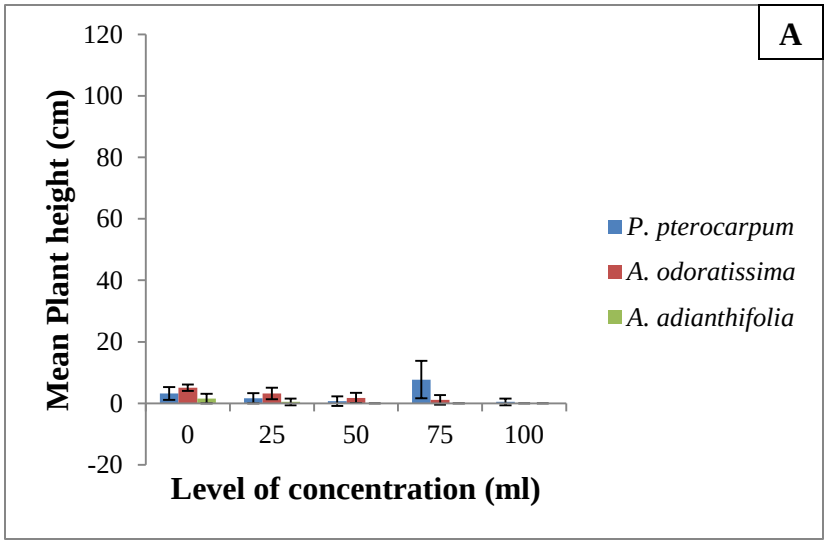
Table 4.2: Correlations coefficients between LTS and crude oil-contamination

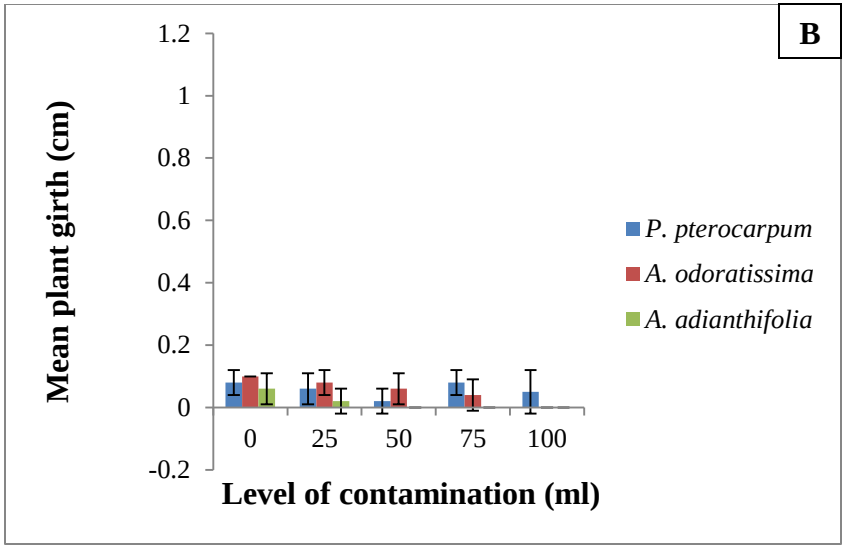
LTS*LoC[†]	**Probability**			
	r	**n**	**P**	**P<0.05**
P. pterocarpum	-0.841	5	<0.001*	
A. odoratissima	-0.875	5	<0.001*	
A. adianthifolia	-0.888	5	<0.001*	

[†]Level of contamination (LoC).

4.2. Evaluation of early growth performance of the selected LTS in crude oil-contaminated soils

Agronomic parameters were employed to determine early growth of the selected LTS in crude oil-contaminated soil under greenhouse conditions over a period of 16 weeks. **Figure 4.3** shows the mean height of the selected LTS planted in crude oil-contaminated soils at 2 WAP. *P. pterocarpum* had mean heights in all the oil-treated soils of 3.22±2.07, 1.68±1.66, 0.70±1.57, 7.72±6.11 and 0.48±1.07 cm in 0, 25, 50, 75 and 100 ml oil-treatments, respectively. The mean girth of *P. pterocarpum* in the uncontaminated soil was 0.08±0.04, while girth ranged between 0.02±0.07 to 0.06±0.05 cm in the crude oil-contaminated soil. At 2WAP, leaf production had not started intensively, with only 1-2 small leaves were produced in the treatments (**Appendix 4.3**). *A. odoratissima* recorded mean height of 5.12±1.03, 3.18±1.87, 1.76±1.65 and 1.10±1.57 cm in 0, 25, 50 and 75 ml crude oil contaminated soil, respectively. There was no growth in this tree species at 2 WAP in 100 ml crude oil contaminated soil. Mean girth was between 0.01±0.00 and 0.08±0.04 cm. The tree species also had low leaf production at this stage and produced only 1-2 small leaves (**Appendix 4.4**). *A. adianthifolia* produced height and girth in 0 and 25 ml crude oil-contaminated soil at 2 WAP. At 2 WAP, mean height was 1.54±1.52 and 0.48±1.07 cm, with corresponding mean seedling girth of 0.06±0.05 and 0.02±0.04 cm, respectively (**Appendix 4.5**).





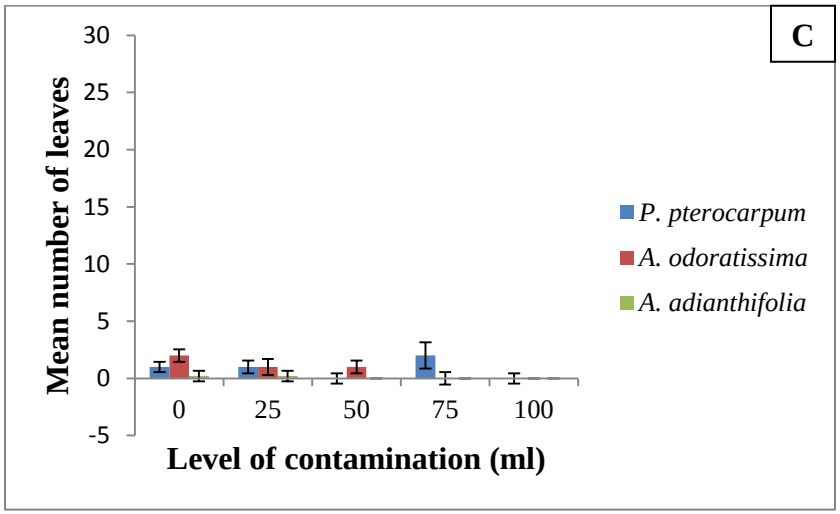
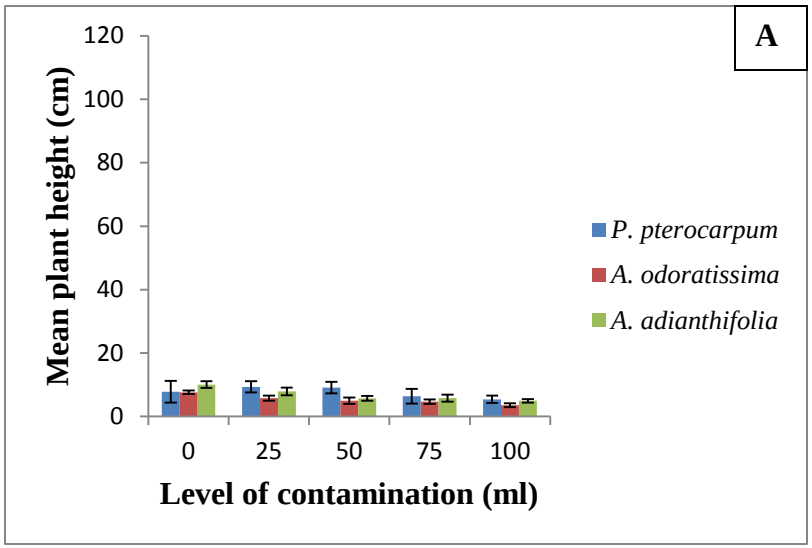
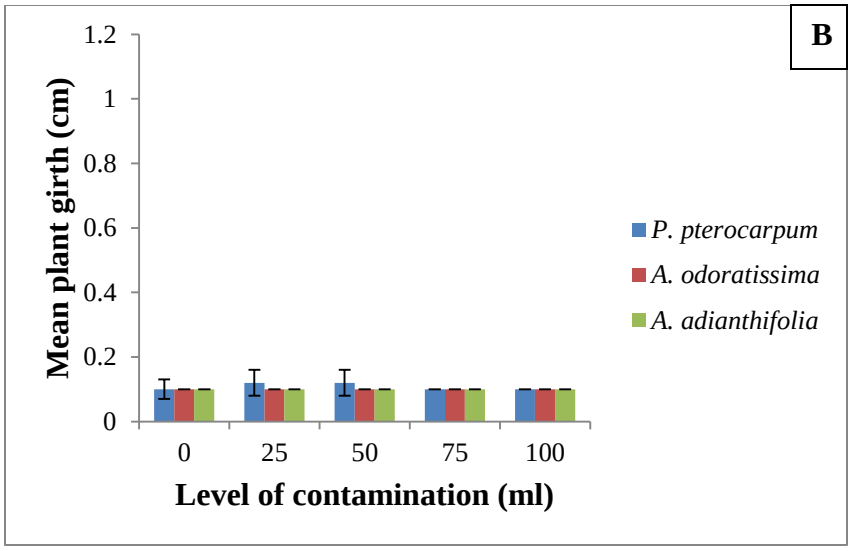


Figure 4.3: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 2 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

Figure 4.4 shows the growth performance of the selected LTS in crude oil-contaminated soil at 4 WAP. *P. pterocarpum* had mean seedling height of 7.84±3.43, 9.36±1.77, 9.16±1.80, 6.38±2.31 and 5.44±1.18 cm in 0, 25, 50, 75 and 100 ml in crude oil contaminated soil, respectively. The mean seedling girth ranged between 0.10±0.00 and 0.12±0.04 cm in the treatments, but seedlings grown in uncontaminated soil had a mean girth size of 0.10±0.03 and so was significantly different from the treatments. Leaf production was 3.00±1.00, 2.00±0.45, 2.00±0.55, 1.00±0.45 and 1.00±0.55 in 0, 25, 50, 75 and 100 ml crude oil-contaminated soil, respectively (**Appendix 4.6**). *A. odoratissima* had mean seedling height of 7.66±0.57, 5.80±0.82, 5.00±0.99, 4.66±0.71 and 3.56±0.58 cm in 0, 25, 50, 75 and 100 ml crude oil-contaminated soil, respectively. However, seedlings did not have significantly different girth sizes. Girth value of 0.01±0.00 cm was observed in all the seedlings produced at this stage, which may in large part be due to the oil-contamination in the soil. In the control experiment, 4.00±1.53 leaves were produced, while 1.00±0.00 to 2.00±0.89 leaves were produced in the oil-treated soils (**Appendix 4.7**). *A. adianthifolia* had mean seedling height of 10.06±1.08, 7.92±1.19, 5.74±0.78, 5.78±1.14 and 4.94±0.54 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. However, seedlings appeared retarded and the mean girth value in the treatments was 0.01±0.00 in both uncontaminated and contaminated soils at 4 WAP. Leaf production in this tree was 3.00±0.71, 2.00±0.71, 1.00±0.00, 1.00±0.00 and 1.00±0.00 in 0, 25, 50, 75 and 100 ml crude oil-contaminated soil, respectively (**Appendix 4.8**).





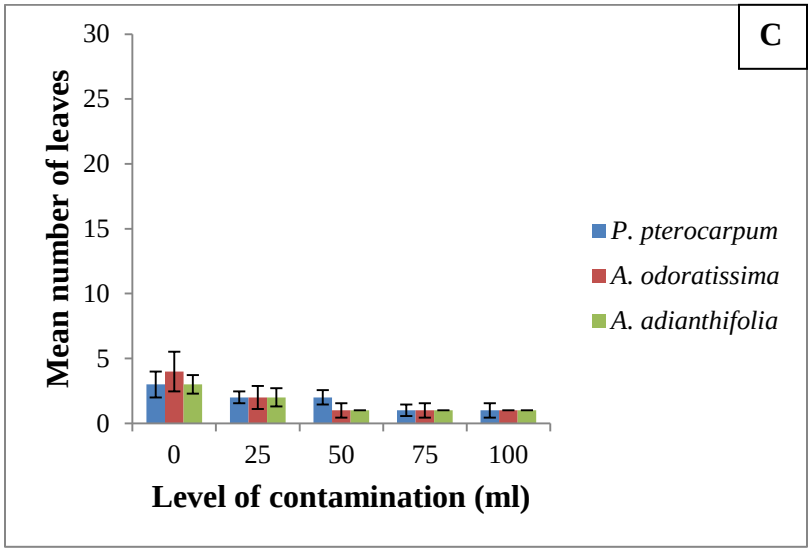
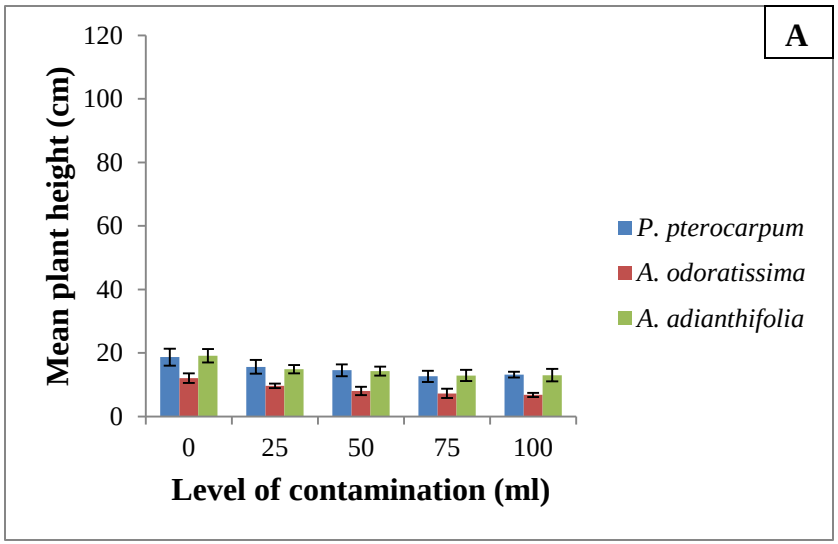
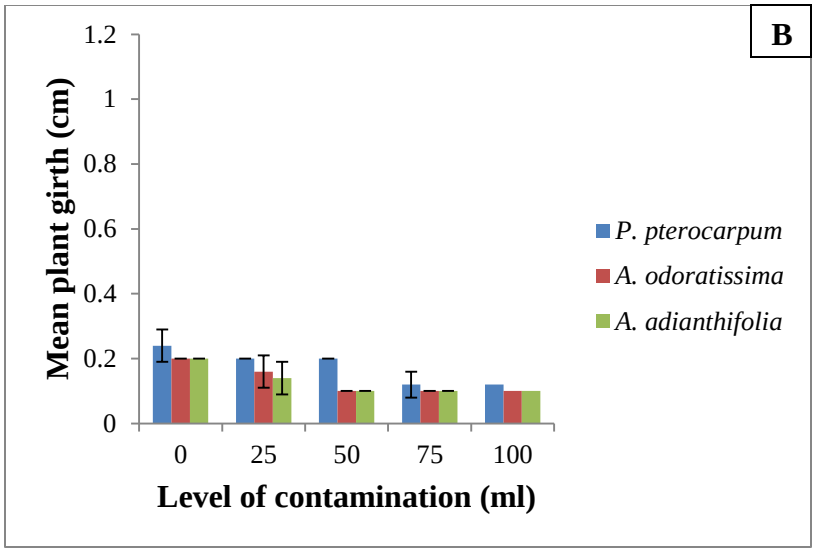


Figure 4.4: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 4 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The growth performance of the selected LTS in crude oil-contaminated soil at 6 WAP is presented in **Figure 4.5**. *P. pterocarpum* had mean heights of 18.70±2.67, 15.64±2.17, 14.54±1.88, 12.64±1.75 and 13.20±0.90 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean girth of 0.24±0.05, 0.20±0.00, 0.20±0.00, 0.12±0.04 and 0.12±0.04 cm were observed in treatments with 0, 25, 50, 75 and 100 ml oil contaminated soils, respectively. Mean number of leaves produced were 4.00±1.30, 3.00±1.00, 3.00±0.55, 2.00±0.45 and 2.00±0.00 in the 0, 25, 50, 75 and 100 ml oil-treated soil, respectively (**Appendix 4.9**). *A. odoratissima* had mean heights of 12.04±1.51, 9.62±0.72, 8.00±1.31, 7.26±1.44 and 6.80±0.64 cm with corresponding mean girth of 0.20±0.00, 0.16±0.05, 0.10±0.00, 0.10±0.00 and 0.10±0.00 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. Leaf production was similar to *P. pterocarpum*. A total of 4.00±0.00, 3.00±0.71, 2.00±0.45, 2.00±0.00 and 2.00±0.00 leaves were produced in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively at 6 WAP (**Appendix 4.10**). Early growth response of *A. adianthifolia* in the greenhouse produced mean seedling height of 19.14±2.10, 14.88±1.30, 14.30±1.42, 12.92±1.75 and 13.02±1.94 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean seedling girth of 0.20±0.00, 0.14±0.05, 0.10±0.00, 0.10±0.00 and 0.10±0.00 cm were observed in the 0, 25, 50, 75 and 100 ml oil-treated soils. *A. adianthifolia* produced 5.00±1.64, 3.00±0.84, 2.00±0.89, 2.00±0.00 and 2.00±0.00 leaves in 0, 25, 50, 75 and 100 ml crude oil contaminated soils, respectively at 6 WAP (**Appendix 4.11**). It was observed that leaf production in the LTS followed a similar trend at 6 WAP.





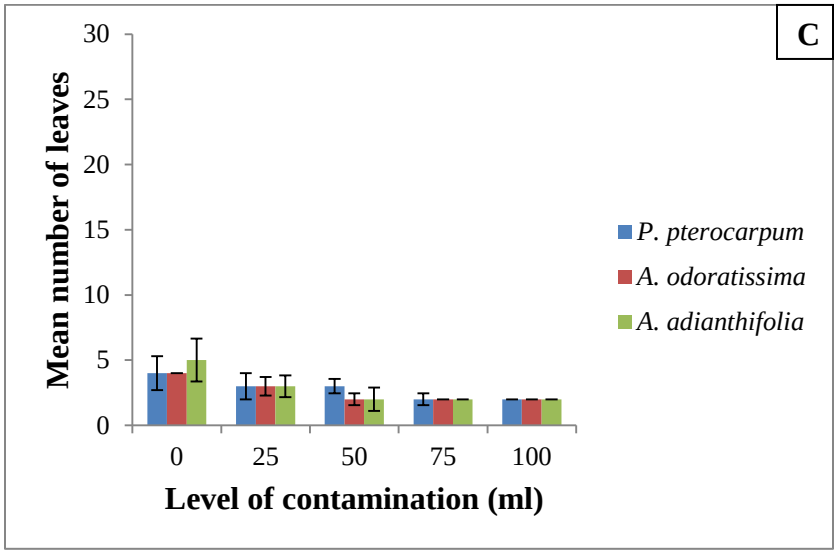


Figure 4.5: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 6 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The growth performance of the selected LTS in crude oil-contaminated soil at 8 WAP is presented in **Figure 4.6**. *P. pterocarpum* had its mean height increased to 28.12±3.56, 24.84±2.77, 23.58±1.58, 20.58±3.22 and 23.00±0.86 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean girth of the seedlings ranged between 0.20±0.00 and 0.26±0.05 cm in the treatments. Mean number of leaves produced were 4.00±1.14, 4.00±0.89, 3.00±0.71, 3.00±0.55 and 3.00±0.45 in the 0, 25, 50, 75 and 100 ml oil-treated soil, respectively, at this age of early growth of the tree species (**Appendix 4.12**). *A. odoratissima* had mean heights of 18.92±1.52, 16.02±1.17, 13.62±0.73, 12.12±0.79 and 10.34±0.61 cm, with corresponding mean girth of 0.20±0.00, 0.20±0.00, 0.18±0.04, 0.12±0.04 and 0.10±0.00 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. Mean leaf production were 6.00±1.10, 4.00±0.55, 4.00±0.55, 2.00±0.00 and 2.00±0.00 leaves in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively, at 8 WAP (**Appendix 4.13**). *A. adianthifolia* growth produced mean seedling heights of 28.44±1.40, 25.56±1.78, 23.18±2.18, 22.68±1.07 and 20.68±2.20 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean seedling girths were 0.22±0.04, 0.20±0.00, 0.12±0.04, 0.10±0.00 and 0.12±0.04 cm in the 0, 25, 50, 75 and 100 ml oil-treated soils and seedling leaf production was 6.00±1.52, 3.00±0.71, 2.00±0.45, 2.00±0.00 and 2.00±0.00 leaves in 0, 25, 50, 75 and 100 ml crude oil contaminated soils, respectively, at 8 WAP (**Appendix 4.14**).

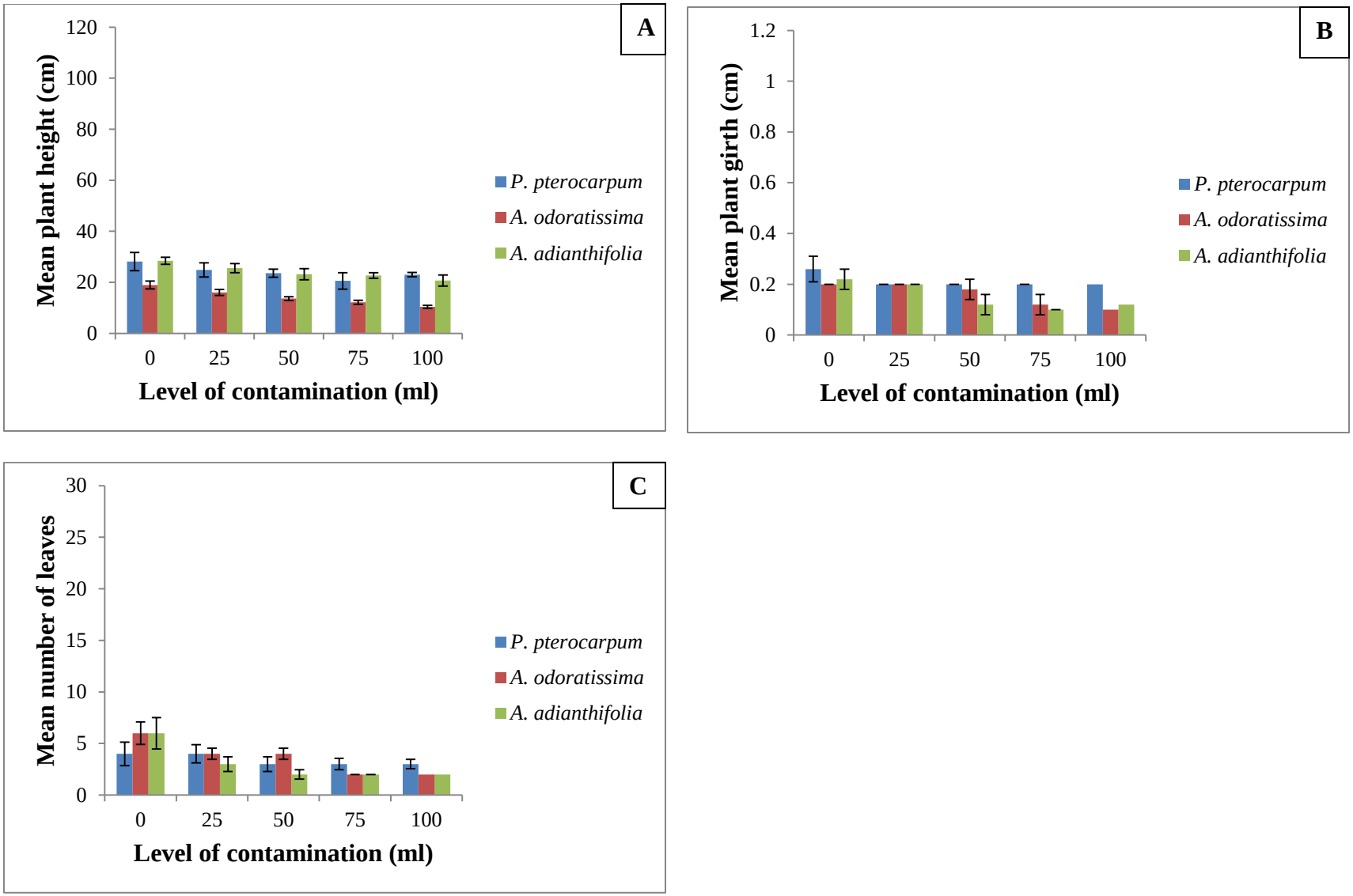
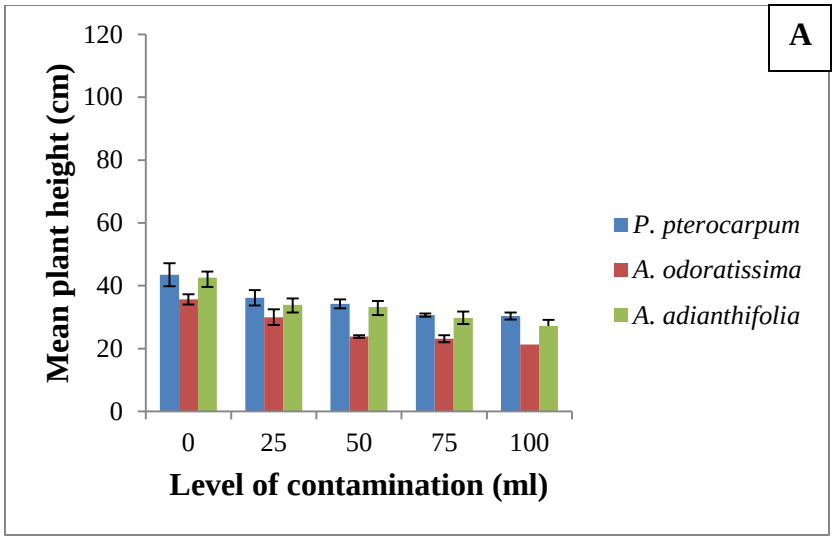
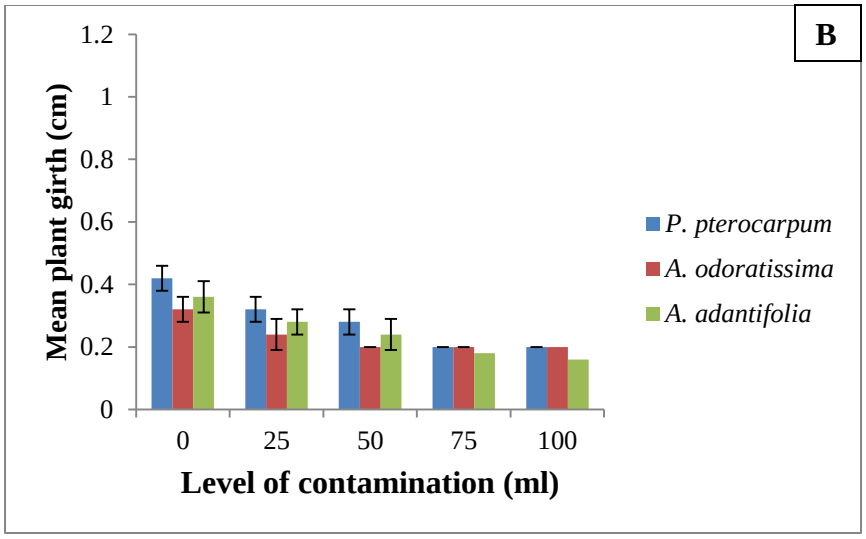


Figure 4.6: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 8 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

Figure 4.7 shows the mean growth performance of the selected LTS in crude oil-contaminated soil at 10 WAP. *P. pterocarpum* mean seedling heights increased to 43.5±3.68, 36.14±2.44, 34.24±1.47, 30.70±0.51 and 30.40±1.10 cm respectively. Mean girth also increased to 0.42±0.04, 0.32±0.04, 0.28±0.04, 0.20±0.00 and 0.20±0.00 cm. The mean number of leaves produced was 7.00±1.87, 6.00±0.45, 5.00±0.45, 4.00±0.55 and 4.00±0.45 in 0, 25, 50, 75 and 100 ml crude oil contaminated soils (**Appendix 4.15**). *A. odoratissima* had mean plant height of 35.64±1.65, 30.00±2.50, 23.88±0.41, 23.18±1.13 and 21.30±0.57 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean girths were 0.32±0.04, 0.24±0.05, 0.20±0.00, 0.20±0.00 and 0.20±0.00 cm, respectively. The mean number of leaves produced by the tree species increased to 6.00±1.00, 4.00±0.45, 4.00±0.55, 2.00±0.55 and 2.00±0.45 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively (**Appendix 4.16**). *A. adianthifolia* had mean seedling heights of 42.56±2.96, 33.98±2.47, 33.22±2.55, 29.78±1.98 and 27.20±3.02 cm, respectively. Mean girths of the seedlings were 0.36±0.05, 0.28±0.04, 0.24±0.05, 0.18±0.04 and 0.16±0.00 cm, respectively. It produced mean numbers of leaves of 7.00±0.71, 4.00±0.04, 3.00±0.45, 2.00±0.00 and 2.00±0.00 in 0, 25, 50, 75 and 100 ml, respectively (**Appendix 4.17**). Thus, the mean growth of plant species was influenced by the concentration of oil in soil (**Figure 4.7**) and there were increases in the studied growth parameters as the experiment progressed.





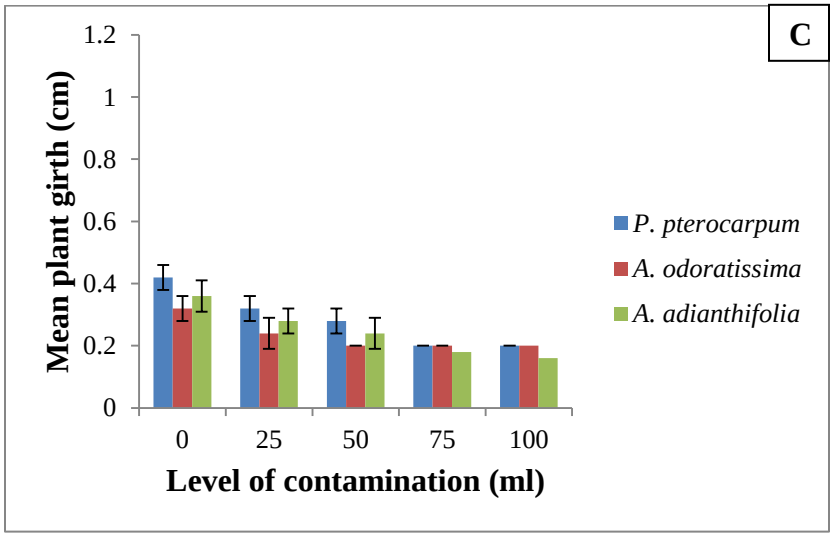
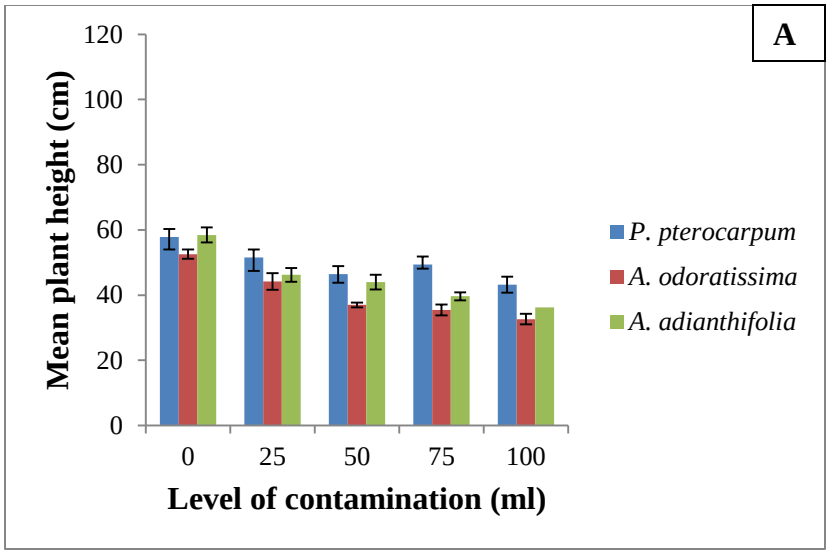
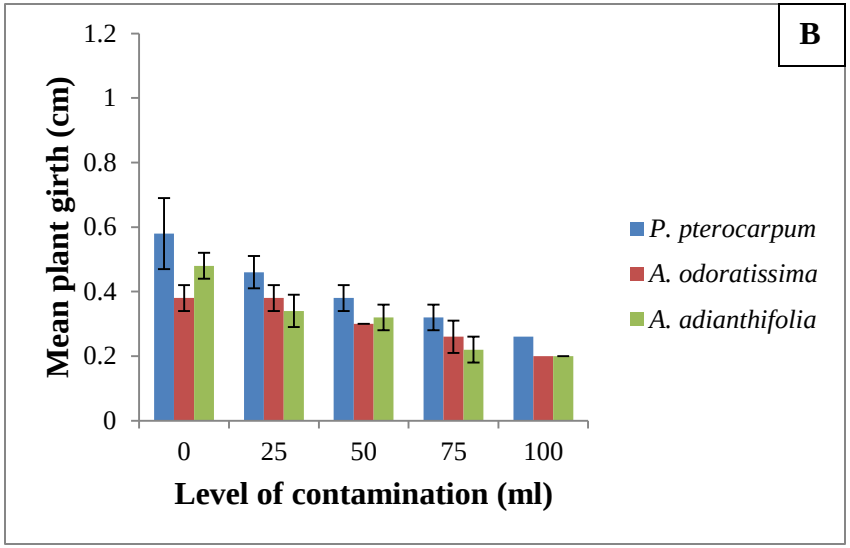


Figure 4.7: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 10 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The growth performance of the LTS in crude oil-contaminated soil at 12 WAP is shown in **Figure 4.8**. The mean height of *P. pterocarpum* increased to 57.80±3.79, 51.56±4.13, 46.42±2.64, 49.34±1.22 and 43.24±2.46 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean girths of the seedlings were 0.58±0.11, 0.46±0.05, 0.38±0.04, 0.32±0.04 and 0.26±0.45 cm in the respective treatments. Mean number of leaves produced increased to 13.00±2.19, 9.00±1.30, 7.00±0.89, 6.00±0.45 and 5.00±0.45 in the 0, 25, 50, 75 and 100 ml oil-treated soil, respectively, at this age of early development of the tree species (**Appendix 4.18**). *A. odoratissima* had mean heights of 52.56±1.39, 44.16±2.57, 36.98±0.71, 35.42±1.69 and 32.62±1.61 cm with corresponding mean girths of 0.38±0.04, 0.38±0.04, 0.30±0.00, 0.26±0.05 and 0.20±0.00 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. Mean leaf production was 8.00±0.89, 6.00±0.55, 5.00±0.45, 2.00±0.55 and 4.00±0.55 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively at 12 WAP (**Appendix 4.19**). *A. adianthifolia* produced mean seedling heights of 58.46±4.86, 58.46±4.86, 44.00±2.08, 39.64±2.28 and 36.26±1.22 cm, with corresponding seedling girths of 0.48±0.04, 0.34±0.05, 0.32±0.04, 0.22±0.04 and 0.20±0.00 cm in the 0, 25, 50, 75 and 100 ml oil-treated soils, respectively. It produced 8.00±0.84, 4.00±0.55, 3.00±0.45, 3.00±0.55 and 3.00±0.45 leaves in 0, 25, 50, 75 and 100 ml crude oil contaminated soils, respectively, at 12 WAP (**Appendix 4.20**). Thus, the mean growth of plant species was strongly influenced by the concentration of oil in soil.





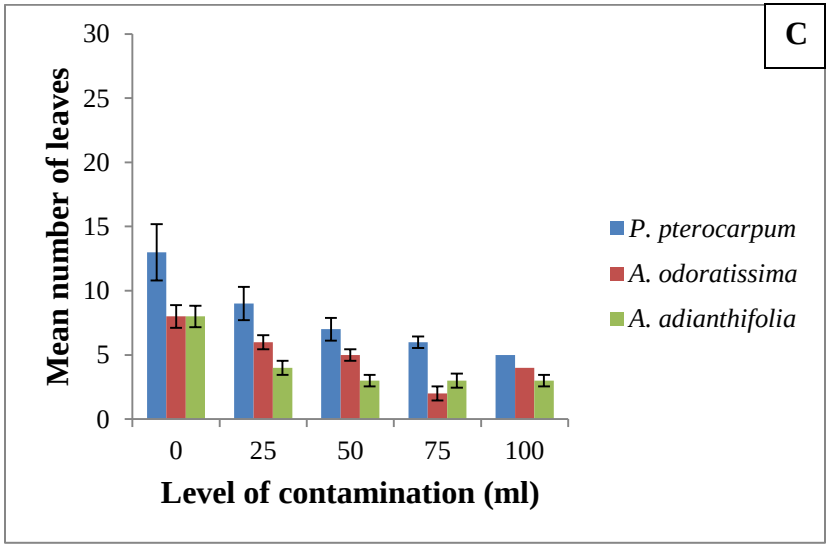


Figure 4.8: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 12 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The mean growth performance of the LTS in crude oil-contaminated soil at 14 WAP is presented in **Figure 4.9**. The mean seedling height of *P. pterocarpum* planted in crude oil contaminated soil was 81.26±3.71, 70.38±3.56, 64.60±3.67, 63.04±2.35 and 56.86±7.28 cm. Mean seedling girth increased to 0.76±0.05, 0.58±0.04, 0.56±0.05, 0.52±0.04 and 0.44±0.09 cm, while the mean number of leaves produced was 20, 14, 7, 11 and 10 in 0, 25, 50, 75 and 100 ml crude oil contaminated soils (**Appendix 4.21**). *A. odoratissima* had mean plant heights of 64.68±3.73, 59.30±1.50, 48.88±1.43, 46.90±0.80 and 43.48±2.77 cm. The mean girths of the seedlings produced were 0.66±0.13, 0.53±0.04, 0.40±0.00, 0.38±0.04 and 0.32±0.04 cm, while the mean number of leaves produced by the tree species increased to 13.00±1.67, 10.00±0.84, 8.00±1.48, 2.00±0.55 and 4.00±0.55 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively (**Appendix 4.22**). *A. adianthifolia* growth produced mean seedling height of 70.80±6.05, 57.96±2.41, 53.58±2.27, 51.44±1.67 and 43.78±1.14 cm in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively. The mean seedling girths were 0.56±0.09, 0.36±0.05, 0.34±0.05, 0.28±0.04 and 0.20±0.00 cm, while 11.00±1.58, 6.00±1.34, 3.00±0.45, 5.00±1.52 and 3.00±0.55 leaves were produced by the seedlings grown in 0, 25, 50, 75 and 100 ml crude oil contaminated soils, respectively at 14 WAP (**Appendix 4.23**). Thus, the mean growth of the plant species was strongly influenced by the concentration of oil in soil in the oil-treatments.

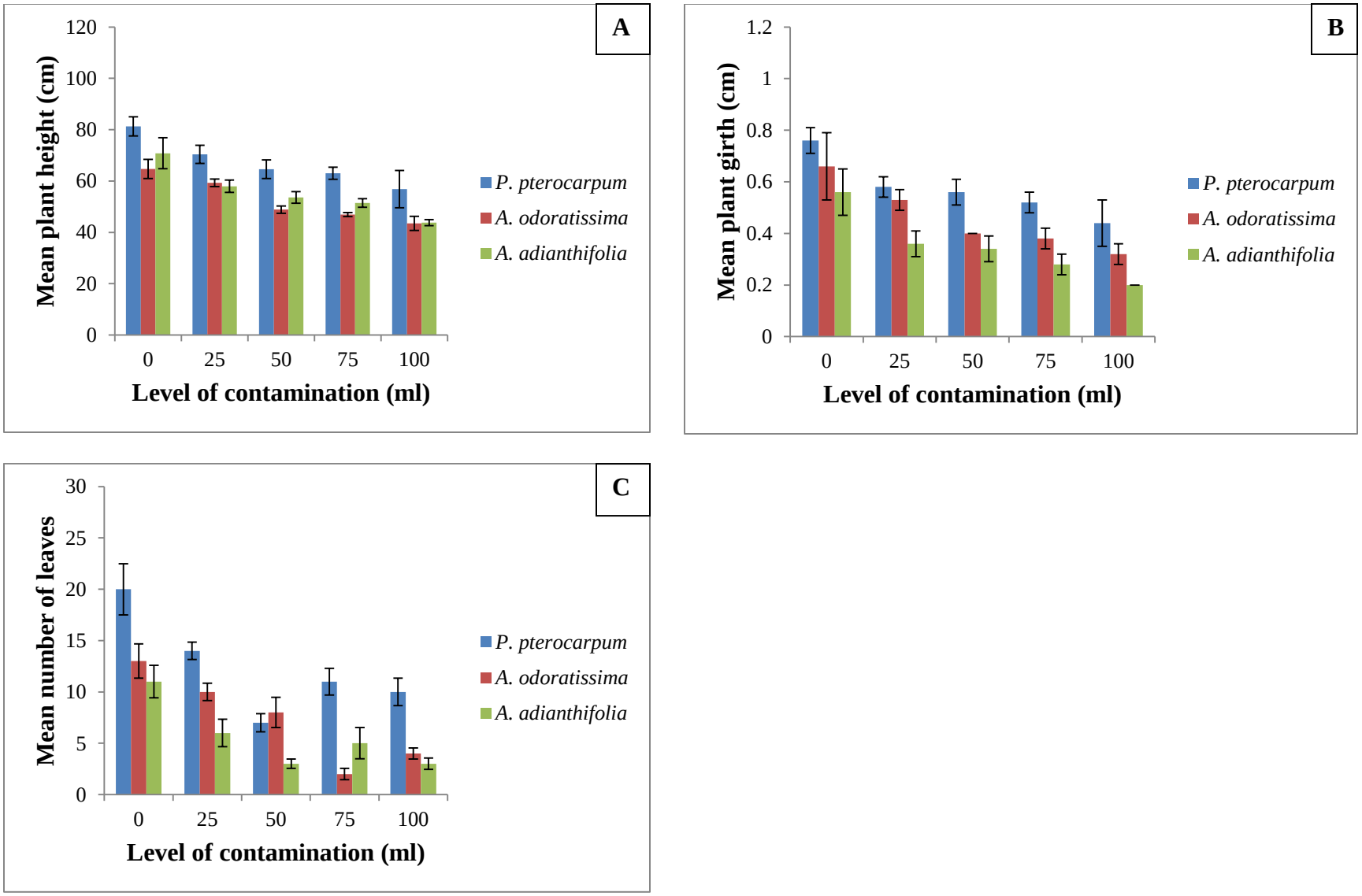


Figure 4.9: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 14 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

The early growth study was terminated at 16 WAP and the mean growth performance of the LTS at 16 WAP is shown in **Figure 4.10**. *P. pterocarpum* had mean heights of 89.58±3.61, 76.68±3.94, 71.38±3.68, 73.28±7.59 and 63.64±7.61 cm, with corresponding seedling girths of 0.82±0.04, 0.68±0.04, 0.62±0.04, 0.58±0.04 and 0.50±0.07 cm, while the mean number of leaves produced was 23.00±2.68, 16.00±1.22, 14.00±1.10, 13.00±1.52 and 11.00±1.64 in 0, 25, 50, 75 and 100 ml crude oil contaminated soils, respectively (**Appendix 4.24**). *A. odoratissima* had mean plant heights of 78.76±5.44, 72.26±2.93, 63.62±3.37, 57.28±1.03 and 54.94±3.33 cm. The mean girths were 0.66±0.05, 0.54±0.05, 0.46±0.05, 0.40±0.00 and 0.32±0.04 cm, while the mean number of leaves produced by the tree species increased to 15.00±2.19, 12.00±1.14, 10.00±0.55, 8.00±0.84 and 4.00±0.45 in 0, 25, 50, 75 and 100 ml crude oil contaminated soil, respectively (**Appendix 4.25**). *A. adianthifolia* had mean plant heights of 81.04±5.88, 68.1±2.42, 63.30±2.94, 60.92±2.90 and 53.76±1.09 cm and mean girths of 0.64±0.09, 0.50±0.07, 0.36±0.05, 0.34±0.05 and 0.24±0.05 cm, while its leaf production increased to 12.00±1.52, 8.00±0.55, 5.00±0.84, 5.00±1.03 and 3.00±0.55 in 0, 25, 50, 75 and 100 ml oil-treated soil, respectively (**Appendix 4.26**). The overall results showed that the mean growth of the plant species was strongly influenced by the concentration of oil in soil and there was an increase in the studied growth parameters with increased age of the experiments. *P. pterocarpum* significantly grown in the contaminated and thus, tended to be most suitable for phytoremediation.

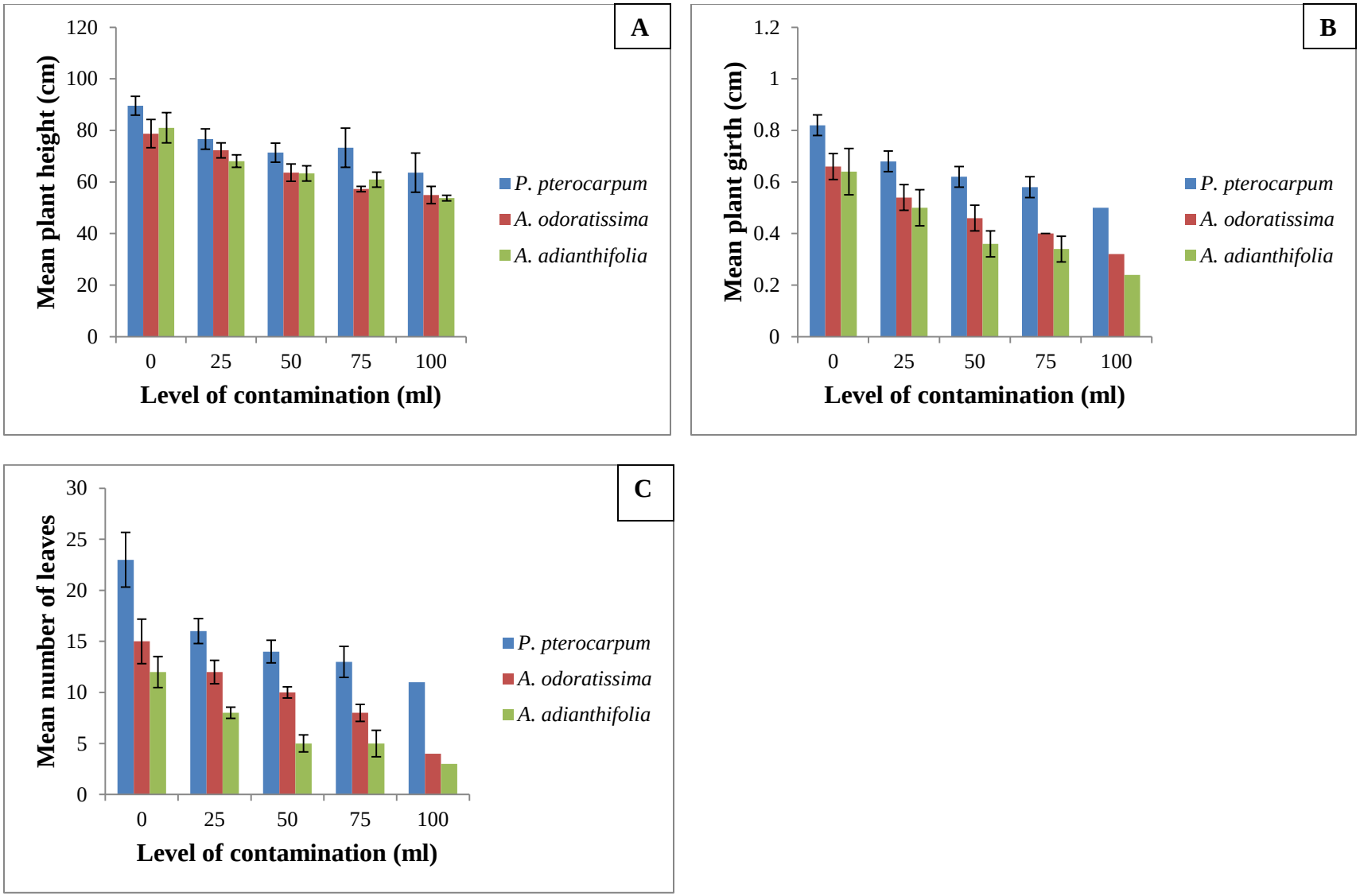


Figure 4.10: Mean (±Standard deviation, n = 5) growth parameters of selected LTS in crude oil-contaminated soil at 16 WAP. [(A) Mean plant height (B) Mean plant girth (C) Mean number of leaves].

Statistical analysis by one-way ANOVA ($P < 0.05$) of growth parameters (height, girth and number of leaves) at 16 WAP showed that the growth parameters of the LTS were significantly different from one another. There were also significant differences at the various levels of contamination among the tree species from those grown in non-contaminated soil (**Appendix 4.27**). Pearson correlation tests between seedling heights and contamination shows that the heights of *A. odoratissima* was the most strongly negatively correlated ($R^2 = -0.930$; $n = 25$; $P < 0.01$) among the LTS. However, *P. pterocarpum* and *A. adianthifolia* were also strongly correlated ($R^2 = -0.792$; $n = 25$; $P < 0.01$) and ($R^2 = -0.911$; $n = 25$; $P < 0.01$), respectively (**Appendix 4.28**). Similar results were obtained for LTS for their girth (**Appendix 4.29**) and leaf production (Appendix 4.30). *A. odoratissima* tended to produce the most significant result in terms of height, girth and leaf production as compared with other LTS. However, the results of *P. pterocarpum* and *A. adianthifolia* were also significant and strongly correlated <0.01. Importantly, *P. pterocarpum* produced more seedlings and vegetative cover when grown in the contaminated soil than any other LTS and this presents it as a good candidate for phytoremediation.

4.3. Nodule production in the selected LTS

Nodule production is one of the prominent attributes of legumes. The microbes in the legumes' rhizosphere are capable of inhabiting the nodules and participating in nutrient cycling. **Table 4.3** shows the number of nodules produced by the LTS grown in the crude oil-contaminated soils in the greenhouse over 16 weeks. Nodule production in *P. pterocarpum* seedlings was significantly higher than the other LTS. It produced a mean

28, 24, 19, 15 and 13 nodules in 0, 25, 50, 75 and 100 ml crude oil-contaminated soil, respectively. *A. odoratissima* produced 18, 15, 14, 10 and 6 nodules in the 0, 25, 50, 75 and 100 ml crude oil-treated soil, respectively. A total of 15, 15, 12, 11 and 5 nodules were observed in 0, 25, 50, 75 and 100 ml treated soil, respectively. *A. adianthifolia* had low nodule production in the highly contaminated soil. The microbes in the seedling rhizosphere may have found the soil conditions too harsh. The overall results of nodule production among the tree species revealed that the number of nodules produced decrease as the concentration of crude oil in the soil increases. This may indicate that indigenous soil microbes can only survive in low crude oil-contaminated soil. The nodules produced by the LTS were also similar to images earlier shown in **Figure 3.13**.

Table 4.3: Mean nodules produced by the LTS grown in crude oil-contaminated soil

LTS/LoC*	**Mean number of nodules**				
	0	25	50	75	100
P. pterocarpum	28	24	19	15	13
A. odoratissima	18	15	14	10	6
A. adianthifolia	15	15	12	11	5

*LoC = Level of contamination (ml).

4.4. Shoot and root biomass of the selected LTS grown in crude oil-contaminated soil

The result of shoot and root biomass of LTS grown in crude oil-contaminated soil shows that *P. pterocarpum* grown in non-crude oil-contaminated soil had mean shoot and root biomass of 48.00±1.95 g and 10.83±1.50 g at 16 weeks, respectively. The seedlings grown in crude oil-contaminated soil had significantly decreased mean shoot biomass of 41.13±2.30, 33.83±1.53, 9.60±1.56 and 7.77±1.78 g. The root system of these seedlings produced mean biomass of 8.67±0.31, 6.93±1.02, 4.03±1.89 and 1.90±0.46 g, respectively in 25, 50, 75 and 100 ml oil-contaminated soil, respectively (**Table 4.4**). The seedlings of *A. odoratissima* produced mean shoot biomass of 42.27±3.53, 39.93±6.27, 22.27±2.04, 8.80±1.83 and 4.93±1.40 g with a corresponding mean root biomass of 8.27±2.00, 5.47±1.14, 3.13±0.35, 2.27±0.42 and 1.53±0.15 g when these seedlings were grown in 0, 25, 50, 75, 100 ml crude oil-contaminated soil (**Table 4.4**). Similarly, seedlings of *A. adianthifolia* grown on crude oil-contaminated soil produced mean shoot biomass of 46.73±1.67, 42.67±1.12, 36.37±1.96, 20.07±1.31 and 10.53±0.70 g, with a corresponding root biomass of 9.33±1.01, 8.27±0.95, 7.03±1.44, 3.03±0.81 and 1.83±0.15 g, respectively in the 0, 25, 50, 75 and 100 ml crude oil-contaminated soil (**Table 4.4**).

The dry matter content of the LTS at the end of 16 weeks growth period provided evidence of high tolerance to oil. All the LTS seedlings were able to tolerate and grow in the crude oil-contaminated soil, but their growth and productivity in terms of shoot and root biomass (dry matter content) were significantly different ($P < 0.05$) (**Appendix**

4.31). Seedlings grown in oil-treated soil had lower biomass as compared with those grown on non-crude oil treated soil and this suggests that the species growth were influenced by crude oil concentrations. However, seedlings grown on soils with 25 and 50 ml (low and moderate) level produced considerably more shoots and roots biomass as compared to shoots and roots produced in non-crude oil-contaminated soils. *P. pterocarpum* showed tolerance to the presence of crude oil in soil at various contamination levels (**Table 4.4**), as it produced more shoot biomass in all the treated soils and the shoots biomass were significantly different ($P < 0.05$) in the treatments. The mean root biomass of *P. pterocarpum* appears best in terms of mean values and tolerance to oil-contamination. It shows no significant differences ($P < 0.05$) in 0, and 25 ml oil-treated soil, but significant differences exist ($P < 0.05$) in 50, 75 and 100 ml oil-treated soil. *A. odoratissima* shoot biomass was not significantly different in 0 and 25 ml oil-contaminated soils, but was significantly different ($P < 0.05$) in 50, 75 and 100 ml oil-treated soil. However, the root biomass was significantly different ($P < 0.05$) in all treatments. Shoot biomass of *A. adianthifolia* shows significant differences for the seedling grown in all the oil-treated soils, but the seedlings root biomass was not significantly different in 0 and 25 ml oil-treated soils, while there were significant differences ($P < 0.05$) in 50, 75 and 100 ml crude oil-treated soils.

Table 4.4: Mean biomass of selected LTS grown in crude oil-contaminated soil 16 WAP* (data represent means ±Standard deviation of 3 replicates)

Treatment (ml)	LTS					
	P. pterocarpum		***A. odoratissima***		***A. adianthifolia***	
	Shoot (g)	**Root (g)**	**Shoot (g)**	**Root (g)**	**Shoot (g)**	**Root (g)**
0	48.00±1.95	10.83±1.50	42.27±3.53	8.27±2.00	46.73±1.67	9.33±1.01
25	41.13±2.30	8.67±0.31	39.93±6.27	5.47±1.14	42.67±1.12	8.27±0.95
50	33.83±1.53	6.93±1.02	22.27±2.04	3.13±0.35	36.37±1.96	7.03±1.44
75	9.60±1.56	4.03±1.89	8.80±1.83	2.27±0.42	20.07±1.31	3.03±0.81
100	7.77±1.78	1.90±0.46	4.93±1.40	1.53±0.15	10.53±0.70	1.83±0.15

*Weeks after planting.

4.5. Results of soil analyses

4.5.1. Physicochemical properties of crude oil-contaminated soil planted with LTS

This section reports the physicochemical properties of crude oil-contaminated soil planted with LTS. The physicochemical properties of the un-contaminated soil used in the experiment were reported in **Table 3.5**. Selected physicochemical soil properties were determined after contamination with varying amount of crude oil and subsequently growing LTS in it over 16 weeks (**Tables 4.5-4.8**). The mean physicochemical properties of crude oil-contaminated soil planted with LTS at 4 WAP are shown in **Table 4.5**. The effect of the presence of oil in the soil was significantly higher as compared to control at this stage and altered both plant growth and soil physicochemical properties. Further, these physicochemical properties were again compared at 8 and 12 weeks after planting (**Tables 4.6** and **4.7**). However, soil nutrient conditions improved as there were more nutrients in the sol at 16 weeks after planting and improved plant growth occurred in the LTS (**Table 4.8**). The LTS treatments were associated with decreased soil acidity. Soil pH in the oil-contaminated soil planted with *P*. *pterocarpum* ranged between 5.71-5.79. Soil planted with *A. adianthifolia* and *A. odoratissima* had values of 6.05-6.34 and 5.56-5.58, respectively, which are weakly acidic as compared with the pH in the soil at 4 WAP when the effect of oil-contamination was high. Soil planted with *P*. *pterocarpum* increased SOM and this may be responsible for the increased seedling growth and high yield biomass. N, P and other soil macronutrients, particularly in the 0, 25 and 50 ml oil treated soil, increased at 16 WAP as compared to uncontaminated soil. The improved soil nutritional conditions may be due to N-fixation

in the root nodules of the LTS and nutrient replenishment through leaf litter and decomposition. Increase in P values may be due to immobilization.

Table 4.5: Mean physicochemical properties of crude oil-contaminated soils planted with selected LTS at 4 WAP[*]

LTS	Treatment (ml)	pH	EC[†] (µScm/cm)	SOC[††] (%)	SOM[†††] (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
P. pterocarpum	Control	5.93±0.02	64.80±0.20	2.27±0.08	3.92±0.14	2.45±0.08	12.17±0.08	32.67±0.17	30.33±0.48	94.23±0.91	78.90±0.54
	25	5.83±0.01	54.30±0.17	2.50±0.08	4.32±0.14	1.47±0.30	10.79±0.71	28.89±0.28	27.84±0.83	64.60±1.59	65.58±0.57
	50	5.87±0.01	46.90±0.89	2.38±0.10	4.19±0.02	1.03±0.16	8.04±0.40	26.14±0.33	26.79±0.33	62.60±2.49	60.86±0.37
	75	5.41±0.18	35.33±0.45	2.02±0.16	3.49±0.26	0.68±0.10	7.76±0.53	24.64±0.14	27.41±1.25	59.21±1.10	61.33±1.40
	100	5.25±0.05	24.43±0.23	1.88±0.05	3.26±0.09	0.47±0.05	5.13±0.26	22.19±0.31	26.29±3.08	52.13±6.81	61.42±0.31
A. adianthifolia	Control	5.13±0.01	34.40±0.53	2.11±0.10	3.64±0.16	2.28±0.04	11.65±0.67	33.03±0.23	30.17±0.46	89.01±0.79	75.79±0.34
	25	5.74±0.01	25.52v0.50	1.75±0.05	3.03±0.09	0.66±0.04	7.70±0.48	30.63±0.50	26.20±1.01	76.06±2.94	60.94±0.74
	50	5.69±0.02	24.93±0.12	1.88±0.03	3.25±0.05	0.62±0.01	7.03±0.47	27.22±0.31	24.66±0.48	66.39±1.30	59.70±0.75
	75	5.55±0.02	33.87±0.21	1.90±0.05	3.28±0.08	0.60±0.02	6.21±0.13	25.02±0.80	23.37±1.63	59.92±1.49	60.16±1.40
	100	5.61±0.01	27.20±0.40	1.80±0.03	3.11±0.05	0.42±0.05	5.05±0.49	23.73±0.25	21.86±0.68	55.49±2.88	60.59±2.06
A. odoratissima	Control	5.84±0.01	25.00±0.26	2.39±0.11	4.12±0.18	2.32±0.12	11.03±0.40	31.50±0.51	30.28±0.15	90.24±1.03	76.62±0.69
	25	5.79±0.01	25.40±0.44	2.18±0.05	3.77±0.09	0.98±0.23	7.17±0.28	30.40±0.96	26.26±1.77	88.41±0.80	60.84±0.61
	50	5.62±0.01	27.30±0.10	0.75±0.08	1.29±0.14	0.80±0.05	6.90±0.61	25.68±0.60	26.88±1.56	85.46±3.04	60.37±0.94
	75	5.44±0.01	42.30±0.20	0.77±0.04	1.33±0.06	0.77±0.04	6.54±0.31	24.37±0.50	26.96±0.44	65.71±2.16	61.33±1.23
	100	5.56±0.01	33.20±0.20	0.78±0.04	1.34±0.07	0.55±0.33	4.35±0.26	21.60±0.44	24.91±0.25	52.69±0.46	60.03±0.43

Data are mean of 3 replicates ±standard deviation.
[*]Weeks after planting
[†]Electrical conductivity
[††]Soil organic carbon
[†††]Soil organic matter.

Table 4.6: Mean physicochemical properties of crude oil-contaminated soils planted with selected LTS at 8 WAP[*]

LTS	Treatment (ml)	pH	EC[†] (µS/cm)	SOC[††] (%)	SOM[†††] (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
P. pterocarpum	Control	5.74±0.02	52.47±0.56	2.39±0.08	4.13±0.13	2.54±0.12	12.76±0.09	33.54±0.44	30.70±0.31	86.80±4.63	80.46±0.63
	25	5.79±0.01	50.70±0.56	2.58±0.01	4.45±0.02	1.56±0.26	12.11±0.57	29.70±0.68	28.43±0.65	64.99±1.21	64.26±1.15
	50	5.76±0.01	43.47±1.78	2.35±0.05	4.07±0.08	0.97±0.12	9.07±0.38	27.87±0.66	27.64±1.90	61.52±0.98	60.90±0.21
	75	5.78±0.01	31.70±0.44	1.80±0.03	3.11±0.05	0.76±0.06	8.37±0.26	25.49±0.36	25.63±2.62	60.70±1.47	60.41±1.23
	100	5.68±0.02	43.27±0.12	1.78±0.05	3.08±0.08	0.58±0.07	5.65±0.39	23.17±0.24	23.36±2.18	53.87±2.04	60.09±1.78
A. adianthifolia	Control	5.72±0.01	45.17±0.06	1.80±0.06	3.11±0.10	2.38±0.09	11.12±1.51	35.47±0.43	30.67±0.31	90.53±0.46	76.97±0.24
	25	6.50±0.01	44.20±0.10	1.77±0.06	3.06±0.09	0.79±0.06	8.57±0.34	34.02±0.30	27.32±0.71	77.97±1.52	59.77±1.34
	50	6.10±0.01	45.77±0.15	1.78±0.06	3.08±0.11	0.66±0.02	7.60±0.32	28.95±0.15	25.42±0.54	72.31±0.67	60.81±1.92
	75	6.13±0.03	40.80±0.26	2.05±0.20	3.55±0.34	0.59±0.03	6.70±0.36	25.66±0.88	24.07±1.71	63.92±1.76	59.79±1.74
	100	6.02±0.02	49.23±0.38	1.75±0.13	3.02±0.24	0.44±0.04	5.73±0.08	24.71±0.40	21.91±0.93	56.73±1.40	59.16±3.14
A. odoratissima	Control	5.83±0.01	32.67±0.15	2.38±0.16	4.11±0.27	2.37±0.08	12.03±0.41	33.51±0.51	31.10±0.33	91.76±2.00	76.76±0.72
	25	5.67±0.00	28.27±0.40	2.12±0.08	3.65±0.14	1.02±0.19	8.20±0.17	28.11±0.34	27.64±1.30	86.34±1.97	59.93±2.20
	50	5.63±0.01	37.40±0.36	1.94±0.06	3.35±0.09	0.92±0.04	7.53±0.31	26.54±0.60	28.23±1.02	82.27±1.16	60.70±1.59
	75	5.51±0.01	32.53±0.15	2.29±0.23	3.96±0.41	0.77±0.06	7.11±0.04	24.94±0.16	27.88±0.68	64.56±0.48	60.43±1.67
	100	5.78±0.01	28.03±0.25	2.18±0.05	3.77±0.09	0.67±0.06	5.24±0.09	24.21±0.74	25.63±2.65	53.52±0.48	61.14±0.75

Data are mean of 3 replicates ±standard deviation.
[*]Weeks after planting
[†]Electrical conductivity
[††]Soil organic carbon
[†††]Soil organic matter.

Table 4.7: Mean physicochemical properties of crude oil-contaminated soils planted with selected LTS at 12 WAP[*]

LTS	Treatment (ml)	pH	EC[†] (µS/cm)	SOC*[††] (%)	SOM[†††] (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
P. pterocarpum	Control	5.64±0.03	57.80±0.26	2.22±0.06	3.84±0.11	2.60±0.07	12.58±0.28	36.20±0.37	31.06±0.51	92.00±0.77	81.04±0.35
	25	5.92±0.02	22.60±0.10	2.19±0.06	3.79±0.10	1.74±0.16	12.10±0.87	32.06±0.60	28.97±0.43	63.58±2.01	67.17±0.98
	50	5.87±0.01	20.50±0.78	1.97±0.12	3.40±0.21	1.07±0.14	10.34±0.42	28.28±0.57	27.97±1.83	61.81±0.64	61.81±1.29
	75	5.82±0.02	35.13±0.38	1.95±0.07	3.37±0.12	0.84±0.12	9.10±0.30	26.11±0.34	26.88±1.10	61.58±1.06	60.19±1.50
	100	5.80±0.02	29.30±0.36	1.78±0.05	3.08±0.08	0.76±0.16	6.32±0.46	24.02±0.80	24.01±1.59	54.87±1.15	60.06±2.28
A. adianthifolia	Control	5.91±0.03	32.00±0.10	1.88±0.05	3.26±0.09	2.44±0.12	10.62±0.89	38.33±0.61	31.47±0.33	91.24±0.77	75.95±0.52
	25	5.80±0.08	31.57±0.06	2.28±0.09	3.95±0.17	0.87±0.03	9.25±0.34	36.31±0.42	27.49±1.42	77.87±0.89	60.17±2.00
	50	5.84±0.07	43.07±0.31	2.40±0.12	4.15±0.20	0.74±0.04	8.28±0.16	31.17±0.24	25.75±0.62	72.95±0.44	60.56±1.99
	75	6.10±0.03	41.87±0.15	2.01±0.07	3.48±0.12	0.64±0.05	6.84±0.06	26.83±0.61	24.36±1.84	63.59±1.56	60.38±2.51
	100	5.83±0.03	48.30±0.17	1.94±0.18	3.35±0.30	0.51±0.05	6.06±0.12	25.12±0.27	22.61±0.46	57.28±0.47	60.49±0.95
A. odoratissima	Control	5.81±0.01	25.13±0.32	2.29±0.04	3.96±0.07	2.46±0.06	11.02±0.88	34.57±0.46	31.84±0.68	94.50±0.39	75.83±0.68
	25	5.85±0.01	28.73±0.35	2.20±0.05	3.80±0.08	1.04±0.19	8.97±0.13	29.83±0.27	28.88±0.73	86.53±0.53	60.29±1.19
	50	5.81±0.02	21.00±0.10	2.19±0.05	3.79±0.08	0.99±0.05	8.36±0.12	27.81±0.30	28.49±0.91	83.30±0.70	60.42±0.28
	75	5.78±0.01	21.37±0.15	1.86±0.10	3.21±0.17	0.88±0.04	7.77±0.09	25.48±0.29	28.43±0.77	65.61±0.72	59.70±0.85
	100	5.73±0.01	25.50±0.10	2.10±0.19	3.64±0.33	0.78±0.04	5.94±0.14	24.96±0.10	26.13±2.62	53.23±1.01	59.83±0.77

Data are mean of 3 replicates ±standard deviation.
[*]Weeks after planting
[†]Electrical conductivity
[††]Soil organic carbon
[†††]Soil organic matter.

Table 4.8: Mean physicochemical properties of crude oil-contaminated soils planted with selected LTS at 16 WAP[*]

LTS	Treatment (ml)	pH	EC[†] (µS/cm)	SOC[††] (%)	SOM[†††] (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
P. pterocarpum	Control	5.65±0.03	37.33±0.35	2.65±0.06	4.58±0.09	2.47±0.08	13.13±0.35	37.87±0.76	32.83±0.66	95.31±0.88	83.04±0.38
	25	5.79±0.04	17.80±0.20	2.51±0.06	4.35±0.10	1.80±0.18	11.38±0.41	34.35±0.52	30.01±0.15	78.14±0.59	68.52±0.44
	50	5.71±0.04	23.90±0.26	2.51±0.05	4.33±0.09	1.12±0.16	10.65±0.74	28.77±0.46	29.21±1.17	67.11±2.45	61.29±0.10
	75	5.76±0.01	23.33±0.06	2.41±0.07	4.16±0.12	0.95±0.09	10.03±0.50	25.22±0.47	27.68±1.01	60.51±1.89	60.58±1.11
	100	5.79±0.04	25.43±0.15	2.11±0.10	3.64±0.16	0.82±0.06	7.23±0.16	24.58±0.45	25.24±0.67	58.38±1.03	59.90±0.45
A. adianthifolia	Control	6.37±0.03	38.70±0.20	2.58±0.01	4.45±0.02	2.54±0.09	10.80±0.74	44.07±0.40	32.19±0.21	92.16±0.56	77.96±0.50
	25	6.05±0.02	30.23±0.76	1.85±0.07	3.21±0.12	0.99±0.03	9.98±0.41	39.83±1.08	28.22±1.42	78.19±0.54	60.17±0.63
	50	6.34±0.01	23.97±0.32	2.33±0.11	4.03±0.19	0.87±0.04	8.77±0.12	34.35±0.38	26.79±0.76	73.66±0.59	61.32±0.22
	75	6.21±0.01	29.17±0.15	1.93±0.08	3.35±0.14	0.71±0.09	7.24±0.16	32.78±0.24	25.10±1.74	64.96±1.05	61.11±1.10
	100	6.11±0.02	39.27±0.31	1.80±0.03	3.11±0.05	0.60±0.05	6.26±0.10	27.11±0.26	23.16±0.25	58.36±1.41	60.92±2.18
A. odoratissima	Control	6.45±0.01	34.87±0.32	2.44±0.13	4.21±0.23	2.53±0.03	10.53±0.31	36.45±0.24	33.56±0.43	94.85±0.70	78.34±0.72
	25	5.61±0.01	40.20±0.30	2.34±0.07	4.04±0.12	1.08±0.16	10.55±0.28	30.89±0.32	29.77±0.55	85.97±0.73	61.84±0.26
	50	5.66±0.01	33.50±1.05	2.31±0.08	3.99±0.13	1.04±0.02	10.36±0.22	28.55±0.42	28.31±0.36	82.18±0.34	61.47±0.42
	75	5.58±0.01	35.63±0.12	1.96±0.09	3.39±0.14	0.94±0.04	8.16±0.07	27.19±0.40	28.99±0.12	65.47±0.96	61.68±1.60
	100	5.56±0.03	31.87±0.06	1.78±0.07	3.08±0.12	0.89±0.07	6.53±0.30	26.03±0.59	28.74±0.67	54.70±0.39	60.93±0.60

Data are mean of 3 replicates ±standard deviation.
[*]Weeks after planting
[†]Electrical conductivity
[††]Soil organic carbon
[†††]Soil organic matter.

4.5.2. Microbial count in the crude oil-contaminated soil planted with LTS

Soil micro-organisms play important roles in plant growth in terms of biogeochemical cycling and hydrocarbon degradation in soil. Microbial populations in *P. pterocarpum, A. odoratissima* and *A. adianthifolia* rhizospheres in the oil-contaminated and non-oil contaminated soils were obtained for each plant species at the end of 16 weeks and compared with the microbial population of the site soil used in the experiment. **Table 3.10** showed that 8.33×10^5 heterotrophic bacteria were observed in the site soil and 3.93×10^5, 8.67×10^4, 7.67×10^4 and 6.67×10^4 were observed in 25, 50, 75 and 100 ml crude oil-contaminated soil, respectively, at 24 hours after contamination. Similarly, a total of 2.37×10^3, 2.50×10^3, 2.43×10^3, 2.17×10^3 and 1.03×10^3 heterotrophic fungi were recorded in 0, 25, 50, 75 and 100 ml crude oil-contaminated soil, respectively, at 24 hours after contamination. Bacteria and fungi were the predominant soil microbial populations but their counts were higher in the site soil than in the oil-treated soils. Oil spillage had the tendency to decrease indigenous soil microbial populations. However, the presence of oil in soil at low concentration had the tendency to stimulate microbial growth (Bamidele and Agbogidi, 2006; Tanee and Kinako 2008).

Table 4.9: Total number of heterotrophic bacteria and fungi (cfu/g) in the rhizosphere of non-contaminated and contaminated soil planted with selected LTS

LoC* (ml)	*P. pterocarpum*		*A. odoratissima*		*A. adianthifolia*	
	HBC (x 10^5)†	HFC (x 10^3)††	HBC (x 10^5)†	HFC (x 10^3)††	HBC(x10^5)†	HFC(x10^3)††
0**	4.27	2.70	2.57	2.47	3.33	2.90
25	7.17	4.13	4.10	2.73	4.70	2.70
50	5.03	3.50	3.93	2.67	4.67	2.50
75	3.40	2.07	2.35	1.97	3.30	1.63
100	2.67	1.40	2.20	1.17	2.87	1.57

*Level of contamination
**Site soil
†Heterotrophic bacteria count
††Heterotrophic fungi count.

The presence and growth of LTS seedlings on the crude oil contaminated soil increased microbial population of the contaminated soil at 16WAP (**Table 4.9**). Crude oil-contaminated soil remediated with *P. pterocarpum* had heterotrophic bacteria counts of 4.27 x 10^5, 7.17 x 10^5, 5.03 x 10^5, 3.40 x 10^5 and 2.67 x 10^5 cfu/g in 0, 25, 50, 75 and 100 ml oil-treated soil, respectively (**Table 4.9**). A total heterotrophic bacteria count of 2.57 x 10^5, 4.10 x 10^5, 3.93 x 10^5, 2.35 x 10^5 and 2.20 x 10^5 cfu/g were in 0, 25, 50, 75 and 100 ml oil-treated soil planted with *A. odoratissima*. Crude oil-contaminated soil planted with *A. adianthifolia* had a bacterial population of 3.33 x 10^5, 4.70 x 10^5, 4.67 x 10^5, 3.30 x 10^5 and 2.87 x 10^5 cfu/g in 0, 25, 50, 75 and 100 ml oil-treated soil at the end of the 16 week period.

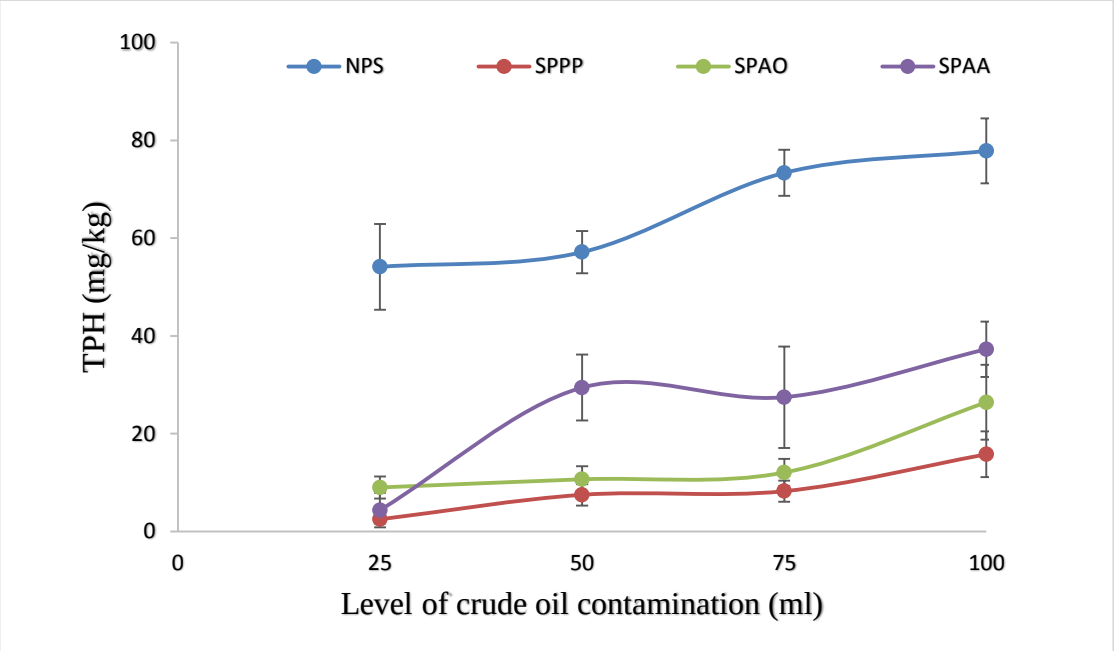
Fungi counts in *P. pterocarpum* were 2.70 x 10^3, 4.13 x 10^3, 3.50 x 10^3, 2.07 x 10^3 and 1.40 x 10^3 cfu/g. A total of 2.47 x 103, 2.73 x 103, 2.67 x 103, 1.97 x 103 and 1.17 x 103 cfu/g heterotrophic fungi were observed in the soil planted with *A. odoratissima*, while a mean fungal population of 2.90 x 10^3, 2.70 x 10^3, 2.50 x 10^3, 1.63 x 10^3 and 1.57 x 10^3 cfu/g heterotrophic fungi were measured in 0, 25, 50, 75 and 100 ml, respectively, in crude oil-contaminated soil (**Table 4.9**). In all the plant-treated oil-contaminated soils, total soil bacteria and fungi counts increased predominantly in the 25 and 50 ml crude oil-contaminated soil as compared with the 75 and 100 ml crude oil-contaminated treatments. Oil in soil may have enhanced microbial growth, particularly at low concentrations, thus the increased population of bacteria and fungi.

These heterotrophic bacteria and fungi are capable of utilizing hydrocarbons and thereby degrade complex hydrocarbon chains to simpler ones. Microscopic examination (**Plate 2.19**) of stained slides prepared from the oil-treated soil showed bacteria and fungi isolates and identification of these isolates were determined using physiological and morphological features of the isolates and compared them with the taxonomic standards (as reported in **Section 3.5.3**). Similarly, bacterial isolates in the oil-treated soils were *Pseudomounas* spp., *Bacillus* spp., *Nocardia* spp., *Micrococcus* spp., *Achromobacter* spp. and *Arthrobacter* spp. The fungal isolates were primarily *Aspergillus* spp., *Fusarium* spp., *Saccharomyces*, *Mucor* spp., *Rhodotorula* spp. and *Rhizopus* spp. These microbes have tendencies for complex hydrocarbon degradation to simple compounds. Statistical analysis by one-way ANOVA (**Appendix 4.32**) showed that mean microbial counts for both soil bacteria and fungal growth were significantly different ($P < 0.05$)

across levels of oil contamination in soil planted with *P. pterocarpum*. Bacterial and fungal populations in *A. odoratissima* and *A. adianthifolia* were not significantly different (P >0.05) across levels of oil contamination. *P. pterocarpum* tended to have the significantly higher microbial populations than other LTS investigated in Experiment II. The microbial populations are capable of degrading hydrocarbons in the crude oil contaminated soil. These microbial populations also have the potential to increase soil fertility.

4.5.3. Hydrocarbon degradation and removal in the rhizosphere of LTS and non-planted crude oil-contaminated soil

The calibrated Gas Chromatography Mass Spectrophotometer (GC-MS) described in **Section 3.4.4** was used for Total Petroleum Hydrocarbon (TPH) determination in the soil treatments. GC-MS showed a TPH in the crude oil of 190.06±0.00 mg/kg. However, oil concentration in the rhizosphere of LTS treatments was considerably decreased as compared to the non-planted crude oil-contaminated soil. The TPH in the various crude oil contamination levels in the LTS rhizosphere and non-planted soil after the 16 week period showed that crude oil compounds were more degraded in the soil planted with LTS than the non-LTS planted soil (**Figure 4.11, Table 4.10**).



NPS = Non-planted crude oil-contaminated soil.
SPPP = Crude oil-contaminated soil planted with *P. pterocarpum*.
SPAO = Crude oil-contaminated soil planted with *A. odoratissima*.
SPAA = Crude oil-contaminated soil planted with *A. adianthifolia*.
Error bars = ±Standard deviation.

Figure 4.11: TPH in the crude oil-contaminated rhizosphere of LTS and non-planted soil at 16 WAP.

Mean TPH in non-planted soil was 54.15±8.76, 57.15±4.29, 73.37±4.71 and 77.86±6.63 mg/kg in 25, 50 75 and 100 ml, respectively, in crude oil-contaminated soil at 16 WAP. There were no significant differences in TPH removal in all non-planted treatments. *P. pterocarpum* planted soil had a mean TPH of 2.50±0.84, 7.51±2.18, 8.256±2.15 and 15.79±4.69 mg/kg in 25, 50, 75 and 100 m, respectively, at 16 WAP (**Table 4.10**). TPH in the *P. pterocarpum* planted soils were significantly different ($P < 0.05$) in 0, 25, 50, 75 and 100 ml crude oil-contaminated soil, which corresponds to the significant decrease in TPH observed in all LTS treatments when compared with the non-planted soil. There were no significant differences in the TPH available in 0, 25, 50 and 75 ml oil-treated

soil planted with *A. odoratissima*, but they were significantly different ($P < 0.05$) in both 100 ml oil-treated soil and non-planted soil. In *A. adianthifolia* planted soil, there were significant differences ($P < 0.05$) among all treatments. TPH available in crude oil-contaminated soil planted with LTS were significantly different ($P < 0.05$) from those in non-planted crude oil-contaminated soil (**Appendix 4.33**).

Pearson correlations between TPH degradation and contamination shows that all the LTS-planted soils were strongly correlated. *P. pterocarpum* planted soil was most strongly positively correlated ($R^2 = 0.818$; $n = 3$; $P < 0.01$) with TPH level among the LTS. However, *A. odoratissima* and *A. adianthifolia* were also strongly positively correlated ($R^2 = 0.731$; $n = 3$; $P < 0.01$) and ($R^2 = 0.746$; $n = 3$; $P < 0.01$), respectively (**Appendix 4.34**). Degradation of the TPH is due primarily to microbial activities in the rhizosphere of the LTS in the crude oil-contaminated soils. Microbial populations in the crude oil-contaminated soil has the potential to influence the hydrocarbon degradation in the crude oil-contaminated soil.

Selected chromatographs of TPH in crude oil-contaminated soils are presented in **Figure 4.12.** Aliphatic hydrocarbon C_9-C_{30} were degraded, while Naphthalene, Biphenylene, Fluorene, Phenathrene and 5H-Indeno[1,2-b]pyridine were the main polyaromatic hydrocarbons degraded in the crude oil-contaminated soils planted with LTS. The rate of TPH degradation was high in the low and moderately oil-treated soils, which could be attributed to the ability of microbes to adapt and survive in such

conditions. Microbial reproduction and respiratory activities may all influence the significant TPH degradation in the low and moderately crude oil-contaminated soils.

There were traces of TPH in oil-contaminated soil detected in the below- and above-ground parts of the LTS, which may have been transferred through plant physiological processes, such as diffusion. Some 0.28-1.65 mg/kg TPH were detected in *P. pterocarpum*, ~0.05-1.41 mg/kg TPH in *A. odoratissima* and ~0.44-1.40 mg/kg in *A. adianthifolia* seedlings grown in the crude oil-contaminated soil. These results indicate these plant species, when grown on contaminated soil, have the potential to take up some contaminants through diffusion. Aliphatic hydrocarbon compounds detected in the plant system are aliphatic hydrocarbons, C_9-C_{30}, but no aromatic hydrocarbons were detected. This is similar to the observation earlier made in **Section 3.4.4**. The presence of these organic compounds in the plant system may alter some physiological processes in plants, such as blockage of respiratory pore spaces and growth retardation, especially at high concentrations of oil. Selected chromatographic peaks of the TPH in plant bodies are presented in **Figure 4.13**.

Table 4.10: Mean (±Standard deviation, n = 3) TPH in crude oil-contaminated soil remediated with LTS at 16 WAP*

Treatment/LTS	TPH (mg/kg) in Non-planted and LTS planted rhizosphere			
	Non-planted soil	***P. pterocarpum***	***A. odoratissima***	***A. adianthifolia***
25	54.15±8.76 (135.91)	2.50±0.84 (187.56)	9.01±2.26 (181.05)	4.38±3.51 (185.68)
50	57.15±4.29 (132.91)	7.51±2.18 (182.55)	10.68±2.66 (179.38)	29.44±6.73 (160.62)
75	73.37±4.71 (116.69)	8.25±2.15 (181.81)	12.07±2.76 (177.99)	27.48±10.38 (162.58)
100	77.86±6.63 (112.20)	15.79±4.69 (174.27)	26.44±7.68 (163.62)	37.30±5.67 (152.76)

*Weeks After Planting (WAP).

TPH degradation over a period of 16 WAP in parentheses.

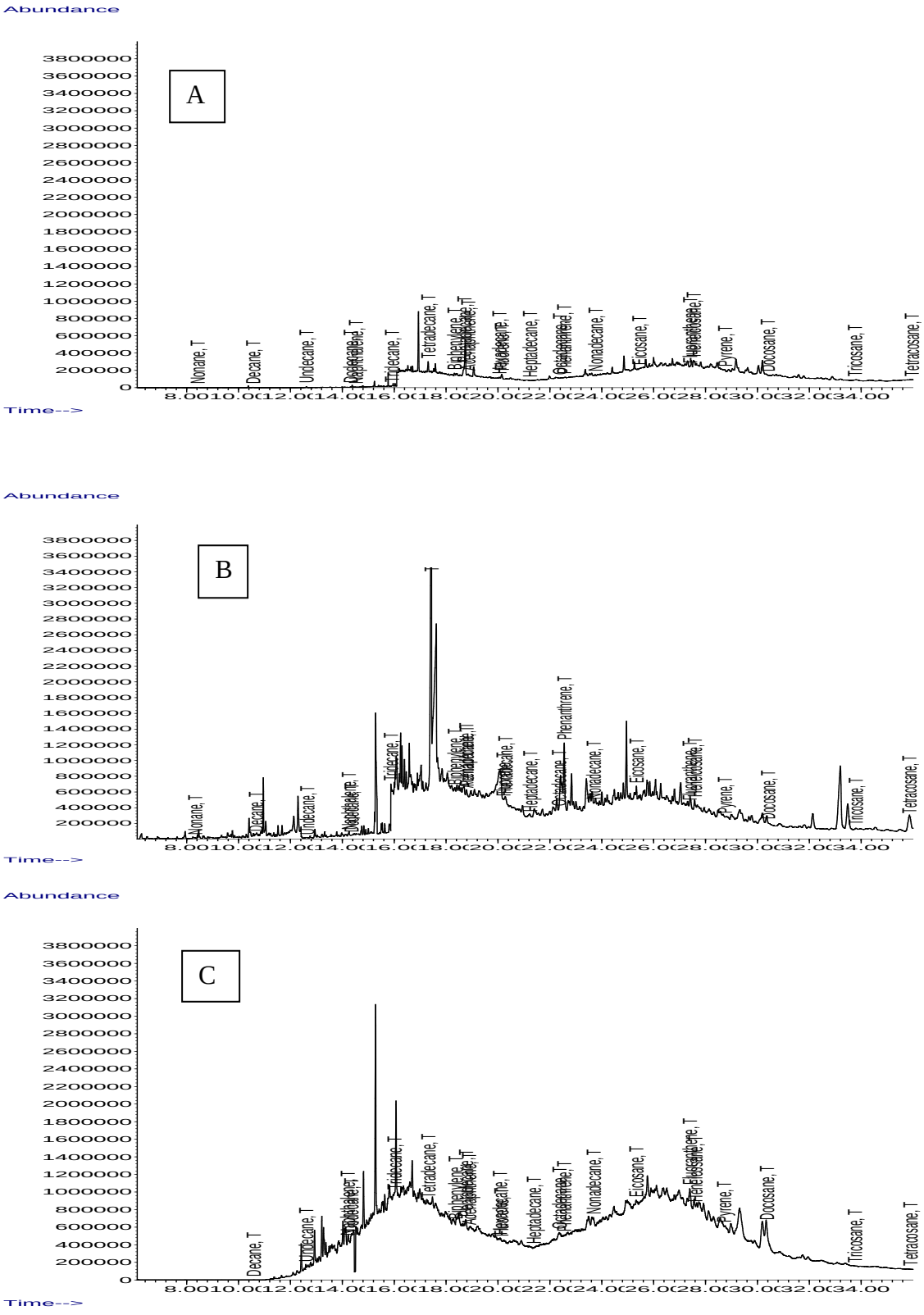


Figure 4.12: Selected chromatographs of TPH in crude oil-contaminated soil: (A) 25 ml crude oil-contaminated soil planted with *P. pterocarpum*, (B) 25 ml crude oil-contaminated soil planted with *A. odoratissima*, (C) 25 ml crude oil-contaminated soil planted with *A. adianthifolia*.

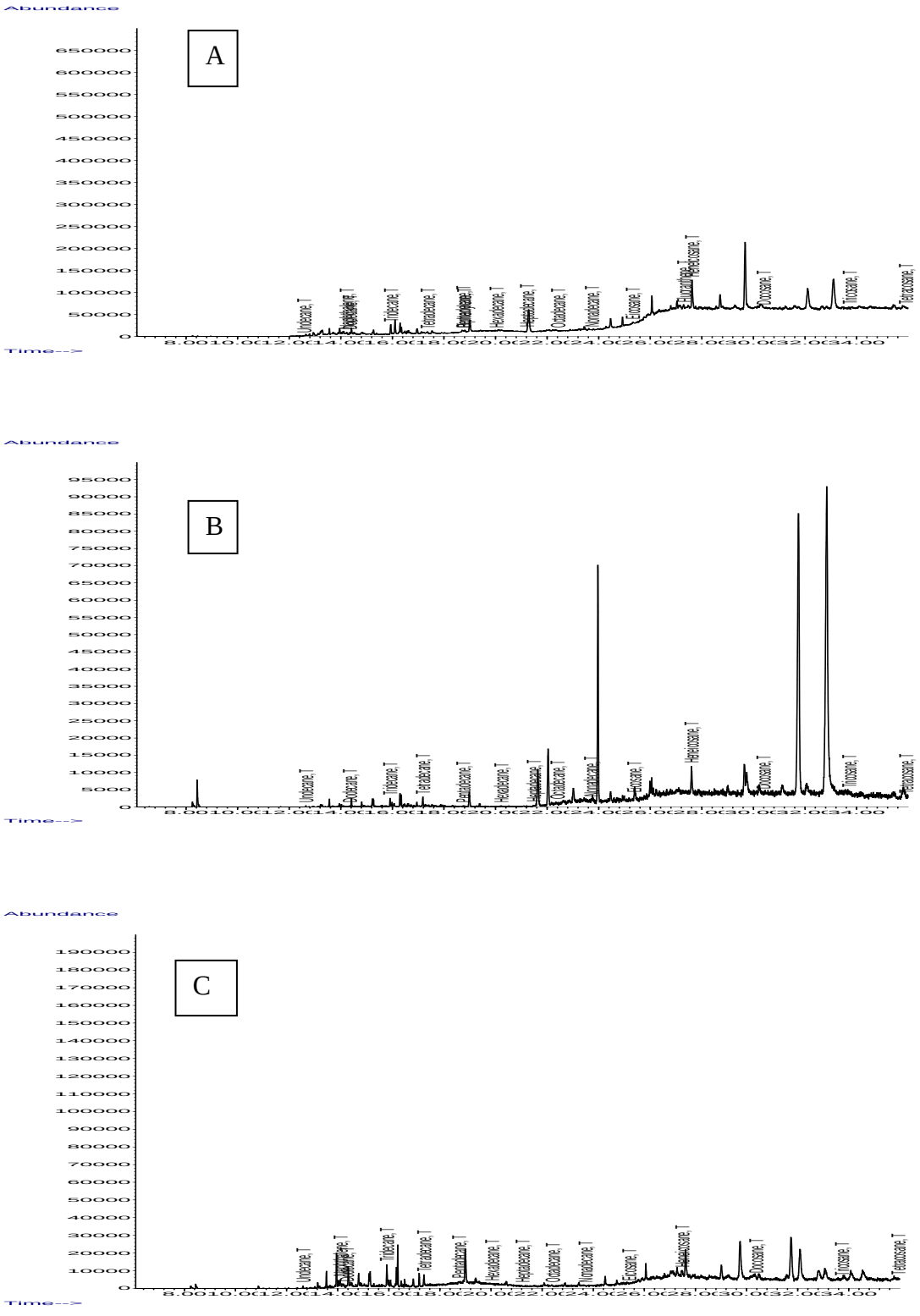


Figure 4.13: Selected chromatographs of TPH in crude oil-contaminated soil: (A) 75 ml crude oil-contaminated soil planted with *P. pterocarpum*, (B) 75 ml crude oil-contaminated soil planted with *A. odoratissima*, (C) 75 ml crude oil-contaminated soil planted with *A. adianthifolia*.

4.6. Conclusions

The success of phytoremediation depends partly on the ability of plants to germinate and establish vegetative growth in oil-contaminated site. Selected plant species should exhibit tolerance in such soil and be able to produce root-soil relationships that will enhance oil degradation in oil-contaminated soil. Selection of plant species for phytoremediation is a critical step, which involves evaluation of germination potential followed by a greenhouse study which can help identify plant species that can tolerate, survive and thrive through the initial establishment period (Bamidele and Agbogidi, 2006). *P. pterocarpum* produced significant germination success with high corresponding COV of seeds germination. Early growth performance in the greenhouse and biomass production was significantly higher than *A. odoratissima* and *A. adianthifolia*. The impact of these LTS on crude oil-contaminated soil also showed that microbial population (which corresponds to hydrocarbon degradation and soil replenishment) were significantly higher in *P. pterocarpum* over other tree species investigated in Experiment II. This species had healthy growth in the oil-contaminated soil conditions and will have a good chance of producing initial vegetation establishment, which will enhance long-term ecosystem processes and oil degradation in oil-contaminated soil.

CHAPTER FIVE

Oil sorption potential of kaolinite

5.0. Introduction

The ability of natural zeolites and clay to act as multi-functional materials in many industrial applications is due to their inherent properties, including uniform pore size, catalytic activity, mobile cation and hydrophilicity/hydrophobicity (Berendsen *et al*., 2006). The efficacy and unique role of natural zeolites and other zeolitic materials for sorption of petroleum products, which constitutes possible environmental contaminants has been identified. The effectiveness of natural zeolites, such as clinoptilolite for sorption of petroleum products (Misaelides, 2011; Obua *et al*., 2014) informed the current study on kaolinite. If clinoptilolite could serve as a good sorbent for petroleum products, then kaolinite may present potential. Kaolinite is a clay or natural zeolite precussor with vast industrial importance and is widely available in Nigeria, and other tropical countries with frequent oil spillages. The remedial usage of kaolinite is therefore tested for oil sorption in this experiment.

5.1. Identification of tropical kaolinite used in the experiment by XRD analysis

The identification of kaolinite was performed using XRD (Reitvield analysis). The kaolinite was mostly crystalline with no amorphous stretch observed around the amorphous regions ~(i.e. between °2Theta 25 and ~45) (**Figure 5.1**).

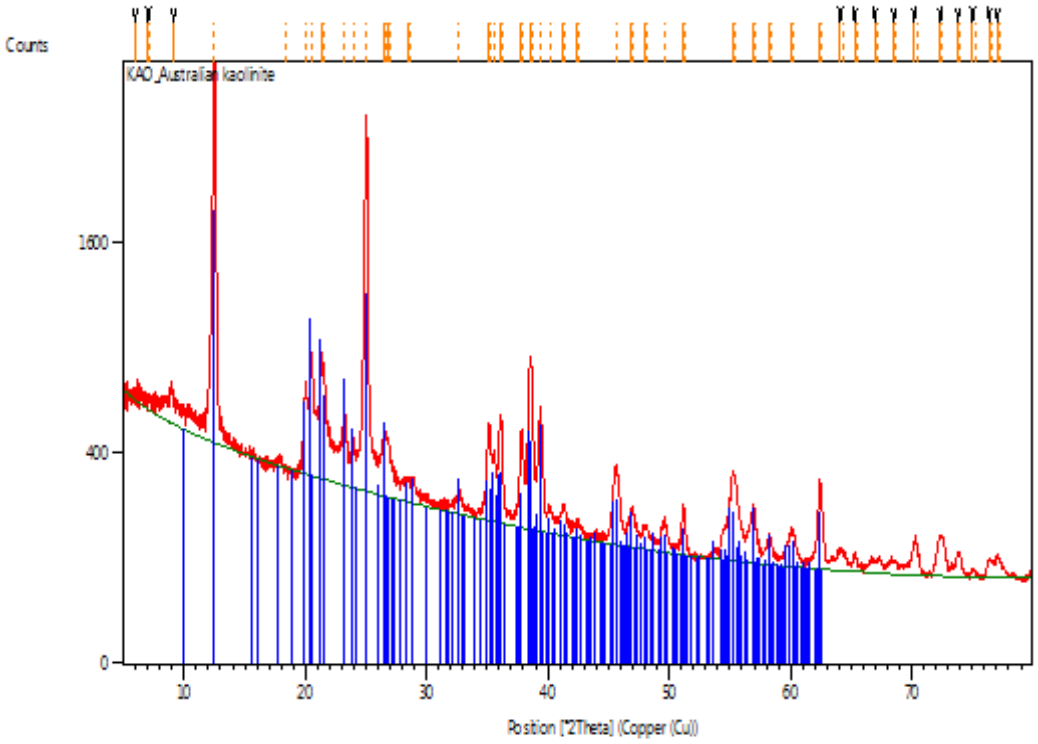


Figure 5.1: XRD analysis of superfine tropical kaolinite.

5.2. Oil sorption by tropical kaolinite

The efficacy of kaolinite (10 and 20 g samples) applications for the sorption of four different amounts of Automobile Oil (AO) (25, 50, 75 and 100 ml), representing low, moderate, high and very high spillages, respectively, was analysed in a laboratory experiment. **Table 5.1** shows that 10 g kaolinite sorbed a mean of 75.28, 74.68, 61.52 and 60.80% of the 25, 50, 75 and 100 ml AO treatments, respectively by 10 g kaolinite. However, in 75 and 100 ml oil-contamination, the rate of oil contamination outweighed the quantity of kaolinite applied, and therefore, sorption decreased. The percentage sorption in the 75 and 100 ml oil contamination was 61.52 and 60.80%, respectively. Oil contamination in these treatments was high and therefore deceased sorption rate.

Table 5.1: Oil sorption using 10 g tropical kaolinite

Oil contamination/spillage (ml)	**0[#]**	**25**	**50**	**75**	**100**
Actual oil sorption by kaolinite (ml)	0	18.82	37.34	46.14	68.80
Sorption by kaolinite (%)	0	75.28	74.68	61.52	60.80

[#]Experiment control.

Increasing the volume of oil contamination resulted in a corresponding increase in the quantity of oil sorbed by the kaolinite. However, the percentage sorption decreased as the amount of oil contamination increased. ANOVA test of oil treatment on 10 g kaolinite using sorption as the dependent variable showed that sorption rate was significantly different among the oil treatments ($P < 0.05$) (**Appendix 5.1**) and comparison of oil sorption rate in the treatments by *posthoc* test (LSD) showed that all the treatments were significantly different ($P < 0.05$) (**Appendix 5.2**). AO contamination and oil sorption in the 10 g kaolinite were strongly negatively correlated ($R^2 = -0.316$; $n = 25$; $P < 0.05$) (**Appendix 5.3**). However, 10 g kaolinite was less effective for sorbing oil contamination at 75 ml and 100 ml and to achieve complete sorption, the quantity of kaolinite was increased to 20 g and the amount of oil contamination was maintained.

The sorption potential of kaolinite progressively increased with the increase in kaolinite to 20 g (**Table 5.2**). Samples of 20 g kaolinite had mean sorption of 93.44, 87.04, 73.49 and 70.86% for 25, 50, 75 and 100 ml AO treatments, respectively, thus oil sorption increased as the amount of oil contamination decreased (**Table 5.2**). However, sorption of the various oil-contamination levels was unique, but the 25 and 50 ml oil-contaminations representing low and moderate contaminations, were most

strongly sorbed. The results recorded for 75 and 100 ml oil treatments improved and, therefore, suggests that the quantity of kaolinite applicable for sorbing oil contamination is proportional to oil contamination

Table 5.2: Oil sorption using 20 g tropical kaolinite

Oil contamination/spillage (ml)	**0**[#]	**25**	**50**	**75**	**100**
Actual oil sorption by kaolinite (ml)	0	23.36	43.52	55.12	70.86
Sorption by kaolinite only (%)	0	93.44	87.04	73.49	70.86

[#]Experiment control.

One-way ANOVA of oil treatment on 20 g kaolinite using sorption as the dependent variable showed that sorption was significantly different among the oil treatments ($P < 0.05$) (**Appendix 5.4**) and comparison of oil sorption rate in the treatments by *posthoc* test (LSD) showed that all the treatments were significantly different ($P < 0.05$) (**Appendix 5.5**). AO contamination and oil sorption in the 20 g kaolinite were strongly negatively correlated ($R^2 = -0.439$; $n = 25$; $P < 0.05$) (**Appendix 5.6**).

The two levels of kaolinite amendment on oil contamination sorbed large amount of oil. The results of the two levels of kaolinite were compared and 25, 50, 75 and 100 ml oil treatment increased the sorption rate by 18.16, 12.36, 11.97 and 10.06%, respectively, when the kaolinite was increased to 20 g **(Table 5.3)**. AO contamination and sorption rate (**Figure 5.2, Appendix 5.7**) in the 10 and 20 g samples were strongly positively correlated ($R^2 = 0.975$; $n = 5$; $P < 0.05$). This implies that the volume of oil sorption is directly proportional to the amount of oil

contamination, but inversely proportional to percentage sorption. It is likely then that further increase in the volume of contamination will result in a peak, as kaolinite will become saturated and an increase in the quantity of kaolinite was thus essential.

Table 5.3: Oil adsorption using 20 g tropical kaolinite

Oil contamination/spillage (ml)	**0#**	**25**	**50**	**75**	**100**
Sorption by 10 g kaolinite (%)	0	75.28	74.68	61.52	60.80
Sorption by 20 g kaolinite (%)	0	93.44	87.04	73.49	70.86
Change in sorption (%)	**0**	**18.16**	**12.36**	**11.97**	**10.06**

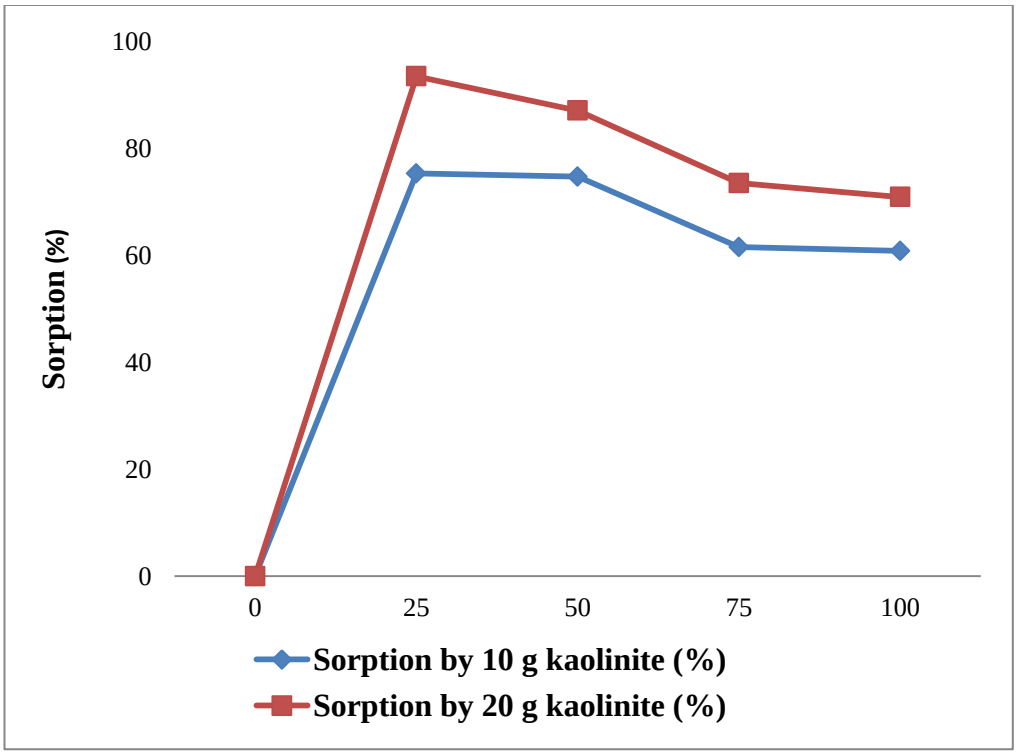


Figure 5.2: Oil sorption 10 and 20 g tropical kaolinite as amendments.

5.3. Re-usability of kaolinite after ignition

The potential re-usability of kaolinite after the initial use for oil sorption was analysed. The highest level of oil-contamination (100 ml) of the two levels of kaolinite amendment (10 and 20 g) was selected and burnt using a Bunsen burner, as reported in **Section 2.10 (Plate 2.23**). The burnt kaolinite was thereafter used for oil sorption following the procedures in **Section 2.10** and it was observed that the kaolinite was re-usable. Kaolinite was still effective for oil-sorption, although, the rate of sorption tended to be less in burnt kaolinite than fresh kaolinite. The 10 g burnt kaolinite sorbed 43.62% while 20 g sorbed 58.90% of the 100 ml of oil. The initial sorption experiment revealed sorption rates of 10 and 20 g fresh kaolinite for 100 ml oil contamination as 60.80 and 70.86%, respectively (**Table 5.4**). The results suggest that the rate of oil sorption by fresh kaolinite is higher than with burnt kaolinite. However, the burnt kaolinite could be potentially re-used for oil sorption.

Table 5.4: Comparison of oil sorption rate of fresh and burnt tropical kaolinite

Kaolinite treatment	Sorption rate (%)	
	10 g kaolinite + 100 ml oil	20 g kaolinite + 100 ml
Fresh kaolinite	60.80	70.86
Burnt kaolinite	43.62	58.90

5.4. Analytical studies on the dry fresh and burnt kaolinite samples

5.4.1. XRD analysis

The identification of kaolinite was performed using XRD. Thr results indicated the sole presence of kaolinite in the powder using Reitvield analysis (**Figure 5.1**). The kaolinite was mostly crystalline with no amorphous stretch observed around

amorphous regions (i.e between °2Theta 25 and ~45). However, XRD analysis revealed that the peaks of both fresh and burnt kaolinites were similar in 10 and 20 g kaolinite samples (**Figure 5.3)**.

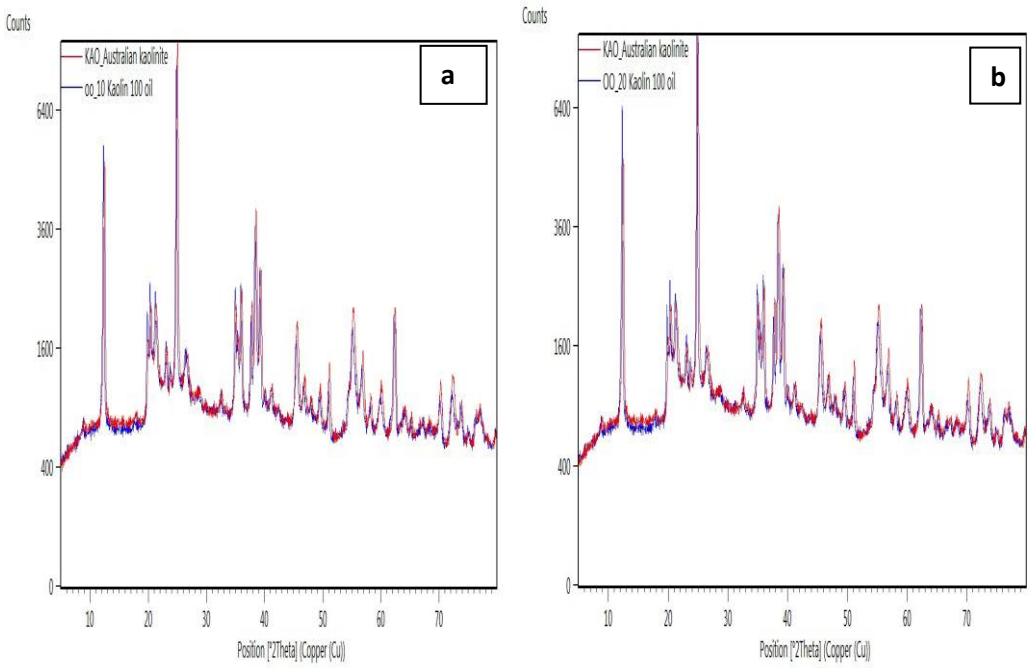


Figure 5.3: Comparison of peaks of elemental compositions of fresh and burnt tropical kaolinite: (a) Fresh and burnt 10 g kaolinite (b) Fresh and burnt 20 g kaolinite sample.

5.4.2. XRF analysis

The kaolinite samples used in the experiment were analysed for their elemental composition and selected elements are presented in **Table 5.5**. The kaolinite samples contain the main elements of zeolites (Al, Si). Kaolinite powder also contains both the macro and micro elements, which could play a vital role in plant growth and productivity yields. Macro elements include Na, Mg, P, K and Ca, while the trace elements include Al, Si, S, Cl, Mn, Fe, Ni, Cu, and Zn. However, XRF analysis revealed some variation in the elemental composition of the fresh and burnt kaolinite samples (**Table 5.5**).

Table 5.5: XRF analysis showing selected elemental compositions of fresh and burnt tropical kaolinite samples

Element	Elemental composition (%)	
	Fresh kaolinite	**Burnt kaolinite**
Na	2.1500	5.5700
Mg	0.2570	0.5510
Al	26.1500	44.1800
Si	36.310	50.3200
P	0.0198	0.1920
S	0.0669	0.1695
Cl	0.0071	0.0161
K	0.5950	0.5710
Ca	0.0573	0.5460
Mn	0.0072	0.0064
Fe	0.3583	0.3667
Ni	0.0057	0.0045
Cu	0.0088	0.0093
Zn	0.0076	0.4196

5.4.3. FTIR analysis

The FTIR spectra of the natural tropical kaolinite used in the experiment is shown in **Figure 5.4** and it is similar to that of natural kaolinite in the FTIR library. Burning did not disrupt the chemical bonding in the kaolinite, as the peak positions of the fresh and burnt kaolinite samples are similar (**Figure 5.5)**.

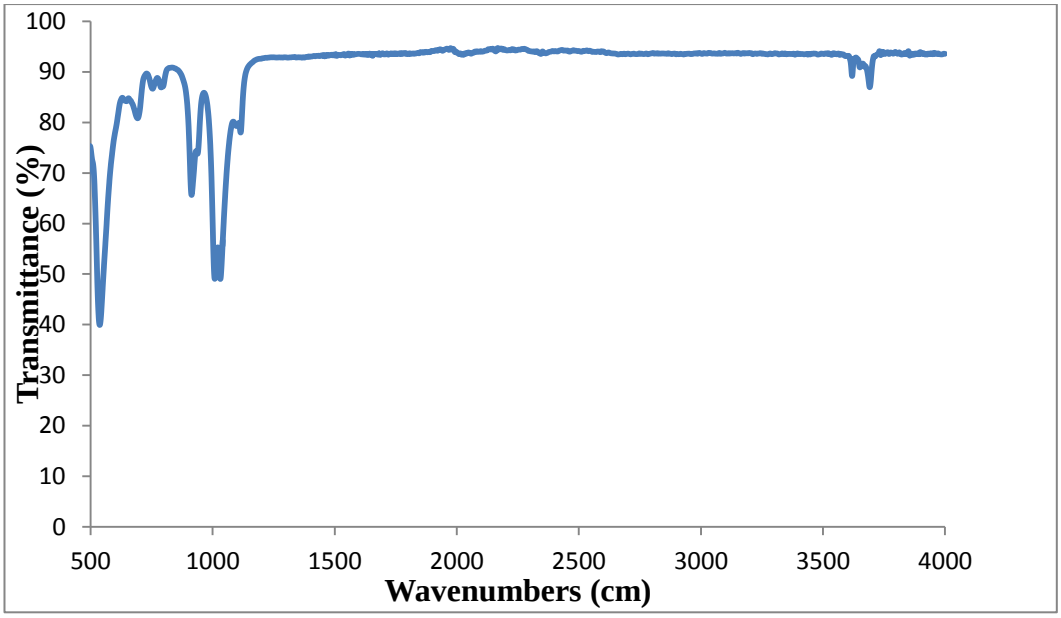


Figure 5.4: FTIR analysis of superfine fresh tropical kaolinite.

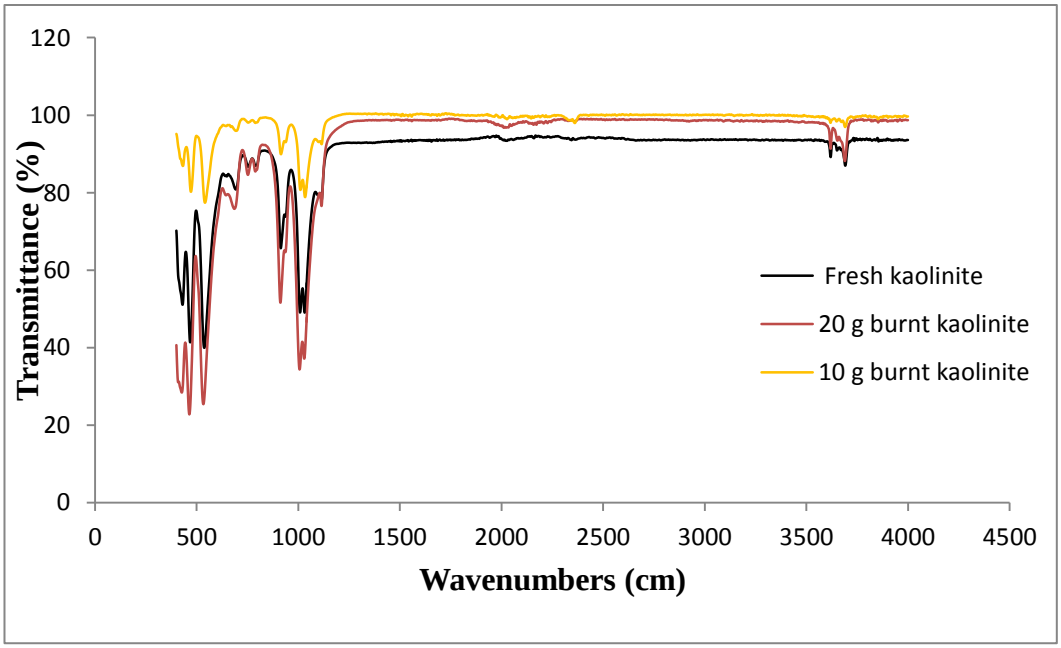


Figure 5.5: Comparative FTIR analyses of the fresh and burnt superfine tropical kaolinite samples.

5.4.4. SEM analysis

SEM images of the fresh and burnt kaolinite samples showed that tropical kaolinite is crystalline (**Plate 5.1**). However, SEM analyses of fresh and burnt kaolinite show distinctive variations. Comparison of images showed some distortions (shown by the red arrows) to the morphological characteristics of kaolinite crystals after burning **(Plate 5.2)** and this may be responsible for decreased oil sorption in burnt kaolinite samples.

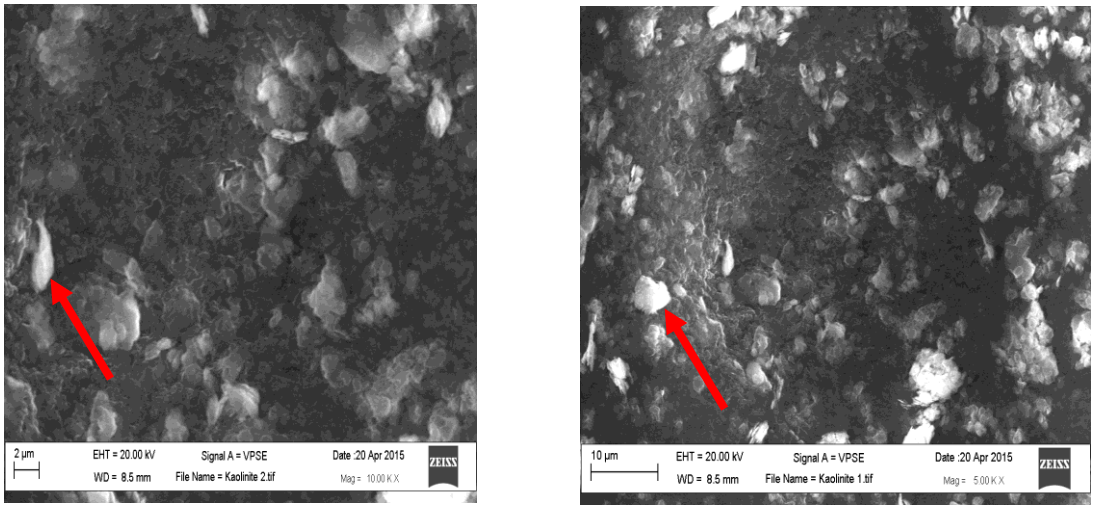


Plate 5.1: SEM images of fresh tropical kaolinite sample.

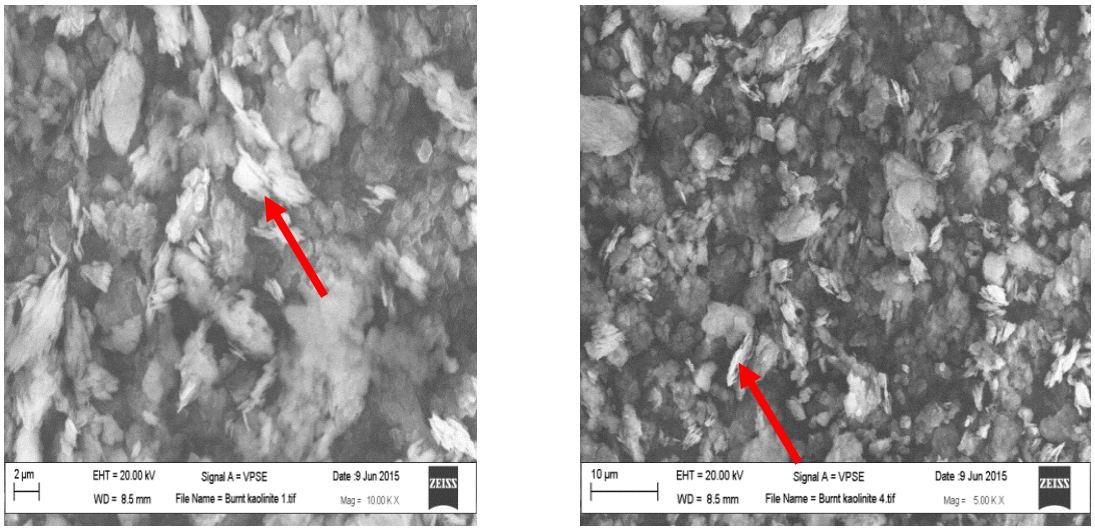


Plate 5.2: SEM images of burnt tropical kaolinite sample.

5.5. Conclusions

Kaolinite is a non-toxic, naturally occurring and abundant clay. It is cost-effective and has higher ion exchange capacity than some tropical natural zeolites (Alpat *et al*., 2008). The experiment provides evidence of the considerable potential of kaolinite to act as a suitable natural cleaning agent and possibly as a soil treatment for partially alleviating oil spills. The results provided similar assertion to Polat *et al*. (2004); Terzano *et al*. (2005) and Obua *et al*. (2014) that natural zeolites are potentially suitable for remediation of contaminated soils, due to their plasticity and high sorption capacity relative to contaminants. Kaolinite found to be suitable and effective for oil sorption. The percentage rate of oil sorption by fresh kaolinite was significantly higher than burnt kaolinite, but burnt kaolinite could be re-used for oil sorption in oil producing countries with crude oil spillage problems. Furthermore, kaolinite could be combined with other environmentally-friendly techniques, such as phytoremediation, to achieve complete removal and remediation of oil-contamination in soils. The results strongly suggest that kaolinite is a potentially environmentally-friendly and suitable 'green technology' for remediation of oil spills.

CHAPTER SIX

General Discussion and Conclusions

6.0. Introduction

Crude oil spillage and its inherent soil contamination as a result of petroleum industry activities and pipelines sabotage is well reported as a frequent occurrence in the oil producing regions of the world, including Nigeria (Osuji and Onajake, 2004; Anoliefo *et al*., 2006a; Brandt *et al*., 2006; Nwaogu *et al*., 2006; Tanee and Kinako, 2008; Peng *et al*., 2009; Aroh *et al*., 2010; Osam *et al*., 2011a; Oyedeji *et al*., 2012). Conventional oil spill clean-up techniques involving physical and chemical processes can further damage soils and ecosystems (Frick *et al*., 1999). Consequently, there is need to develop an environment-friendly technique that will not only degrade hydrocarbon contaminants in soil, but also restore terrestrial ecosystems.

Phytoremediation is an aspect of biological remediation (bioremediation) strategies that involves the use of living plants, soil amendments with associated microbes in plant root systems and agronomic practises for the removal, degradation, extraction and detoxification of contaminants (both organic and inorganic in soils, sediments, air and groundwater) (White *et al*., 2006). It is a non-destructive, cost-effective *in situ* technology that uses plants and their associated micro-organisms to clean up contaminated soils and it is, therefore, appropriate and useful in cleaning up contaminants from environmental systems (Nie *et al*., 2011). It has become a practicable solution for remediation of petroleum hydrocarbon polluted sites (Tanee and Kinako, 2008; Liao *et al*., 2015), not only in developed counties, but also in developing nations with vast oil resources (Zand *et al*., 2010). Synergistic

relationships of plant root systems and soil microbial populations promote degradation of petroleum hydrocarbons in phytoremediation processes (Zand *et al*., 2010). The relationship between plant root systems and soil microbial populations enhances the breaking down of complex hydrocarbons to simple and non-toxic compound throught rhizodegradation process. Removal of petroleum hydrocarbons from soil compartments in phytoremediation works is often attributed to microbial activities in the rhizosphere under the influence of plant root systems (Joner *et al*., 2006). Plants that can tolerate and grow well in crude oil contaminated soil are potentially suitable species for phytoremediation of crude oil-contaminated soil (Tesar *et al*., 2002; Bamidele and Agbogidi, 2006).

Earlier phytoremediation research (Anoliefo 2006a; Chiapusio *et al*., 2007, Tanee and Kinako, 2008; Al-Awadhi *et al*., 2009; Njoku *et al*., 2009; John, *et al*., 2011; Ogbulie *et al*., 2011) focused on the use of leguminous herbs and shrubs, some of which are edible plant species with short growth life spans. However, to prevent inherent dangers of their use on health and food security in oil producing countries, the present study was based on the use of leguminous tree species (LTS). These will serve as nutritional scaffolds for microbial populations to achieve hydrocarbon degradation and environmental clean-up, and present economic value in terms of timber production and other uses. Therefore, impacts of selected LTS in the enhancement of bioremediation of crude oil-contaminated soil in the plant rhizosphere were investigated. The potential of superfine tropical kaolinite as a suitable sorbant and pre-amendment treatment of oil-contaminated soil was also examined. This work covered assessment of different levels of contamination (0, 25, 50, 75 and l00 ml in 4000 g soil; which represents the degree of light crude oil

spillage as 0.0, 0.63, 1.25, 1.88 and 2.5 %v/w) on the biological performance of the investigated LTS (*Albizia adianthifolia*, *Albizia odoratissima*, *Bauhinia monandra*, *Delonix regia*, *Peltophorum pterocarpum* and *Tetrapleura tetraptera*). These LTS were investigated in two phases. The first phase included, *B. monandra*, *D. regia* and *T. tetraptera*, whilst second phase included, *A. adianthifolia*, *A. odoratissima* and *P. pterocarpum*.

Each species was studied for germination success and moistened with varying crude oil-contaminated soil water extracts. Early growth performance in the greenhouse was assessed, in terms of seedling height, girth and leaf production, nodulation and plant biomass production. Soil physicochemical and microbial properties (un-contaminated, contaminated and planted with LTS), total petroleum hydrocarbon (TPH) degradation in the rhizosphere and impact of the species on selected soil physicochemical properties was assessed. The establishment of LTS seedlings in the field was also investigated. Results presented in this work indicate variations in germination tests, biological performance and soil recovery among the LTS in the various oil-treatments. The results have also made it possible to determine the suitability of the LTS for agroforestry bioremediation. The results of the kaolinite experiment have shown the oil sorption potential of kaolinite and its possible role in the remediation of oil spills. The results indicate kaolinite is a suitable pre-amendment treatment for oil-contaminated soil prior to phytoremediation. The overall results obtained in the plant and kaolinite experiments are systematically discussed under the following headings.

6.1. Percentage germination of selected LTS moistened with varying concentrations of crude oil-contaminated water extracts

The ability of plant species to germinate and establish plant cover on crude oil-contaminated soil is crucial to selection of plant species for phytoremediation. In considering a particular species for remediation, the selected plant species should exhibit germination success, tolerance in crude oil-contaminated soil and suitable root-soil relationships, which will enhance microbial growth for oil degradation in oil-contaminated soil. The screening of selected plant species for germination success and growth in crude oil-contaminated soil is one of the initial steps in the selection of plants for successful phytoremediation. This step is considered prior to use of LTS for hydrocarbon degradation in the rhizosphere. However, germination success alone do not account for the growth of plant species on crude oil-contaminated soil (Smith *et al*., 2006). The germination of some plant species may not be affected in oil-contaminated soil, but subsequent growth may be adversely affected and therefore, germination test alone does not give complete information to assess species suitability. A potential promising plant species for phytoremediation is expected to show adequate germination success and minimal adverse growth effects in the presence of contamination (Tesar *et al*., 2002; Merkl *et al*., 2004b).

The germination of the selected tree species, *A. adianthifolia*, *A. odoratissima, B. monandra, D. regia, P. pterocarpum* and *T. tetraptera* when moistened with crude oil-contaminated soil water extracts showed that their germination rates varied and were altered by the presence of oil in soil extracts. However, all tree species germinated when moistened with crude oil contaminated soil water extracts of varying concentrations, but percentage germination decreased with increased amount

of oil in soil water extracts (**Sections 3.1** and **4.1)**. This highlights the importance of plant screening prior to use for phytoremediation, to confirm species suitability and determine seed quality. Variation in seed germination can be attributed to species inability to establish from seeds as other species and this may be due to inherent problems related to seed quality and seed dormancy (Adkins *et al*., 2002). This emphasizes the importance of seed pre-treatments, seed viability and dormancy checks through methods such as seed scarification, soaking seeds in water over a period of time which has proven success of breaking seed dormancy in plant species (Anoliefo and Vwioko, 1995; Adkins *et al*., 2002; Oyedeji *et al*., 2015b). This may further be an option for future optimization of germination success in phytoremediation practise using these LTS for *in situ* field experimentations in the tropical oil-producing nations where they are native.

Successful germination of seeds in crude oil fraction-diesel fuel contaminated soil is highly dependent on plant species and concentration. Some species are notably tolerant (Smith *et al*., 2006), whilst other species are completely intolerant of diesel contamination (Adams and Duncan, 2002). The variability in tolerance was evident among the LTS studied in this research. Some showed no significant adverse effect on seed germination, particularly at high crude oil concentrations when compared with the control. This demonstrates a wide range of tolerance to crude oil-contamination. Similarly, germination studies on alfalfa (*Medicago sativa*) and fescue (*Cyndon dactylon*) showed decreased percentage germination and a relative increased percentage germination for *M. sativa* and *C. dactylon*, respectively in oil-contaminated soil (Al-Ghazawi *et al*., 2005). Germination was studied on filter papers, like the current study, so results would only be a preliminary indication of oil

inhibition of seeds under real soil conditions. Grasses and legumes are tolerant to hydrocarbon contaminated soil when seed germination was studied for 10 days germination periods in hydrocarbon contaminated soil (Smith *et al*., 2006).

Germination was significantly different in the treatments and among the LTS and similar observations were made for their corresponding COV. In the treatments, low oil-treatments had higher germination rates than high oil-treatments, thus the low tolerant species may have struggled to survive in such high oil contamination tretments. Percentage mean germination in *D. regia* seeds (**Table 3.1, Appendix 3.2**) and *P. pterocarpum* seeds (**Table 4.1, Appendix 4.2**) were more strongly negatively correlated with contamination than other LTS moistened with the same extracts in **Chapters 3** and **4**, respectively. The results obtained in this study aligned with earlier research reporting that high concentrations of oil in soil usually stunt the germination and growth of most plant species (Amakiri and Onofeghara, 1983; Anoliefo and Okoloko, 2000; Osuji *et al*., 2005; Kayode *et al*., 2009a), but some plants can withstand some oil contamination (Osam *et al*., 2011, Tesar *et al*., 2002; Ogboghodo *et al*., 2004; Oyedeji *et al*., 2015b) and grow in oil-contaminated soils. Some plants grow well in oil-contaminated soil and such plants are potentially suitable species for the phytoremediation of crude oil-polluted soil (Bamidele and Agbogidi, 2006). This suggests that these LTS species, particularly *D. regia* and *P. pterocarpum* (and to a lesser extent *B. monandra* and *A. odoratissima*) demonstrated promising results as being suitable for phytoremediation of crude oil-contaminated soils, having shown significant germination success in the presence of crude oil-contamination (**Sections 3.1 and 4.1**).

6.2. Evaluation of early growth performance of the selected LTS in crude oil-contaminated soils

Seedling height, girth and leaf production are important agronomic parameters used in the determination of plant health in the medium on which it is grown. Oil contamination of agricultural soils generally decrease plant growth and productivity (Anoliefo *et al*., 2006a; Kayode *et al*., 2009a; Zand *et al*., 2010); although, growth in some plants is possible with low levels of oil contamination (Amakiri and Onofeghara, 1983; Tesar *et al*., 2002; Osam *et al*., 2011a, Liao *et al*., 2015; Oyedeji *et al*., 2015b). However, reasons for reduced plant growth are numerous and include inhibitory and direct toxic effects of oil on plants (Amadi *et al*., 1996; Kroening *et al*., 2001), lack of viable seeds (Rowell, 1977; Adkins *et al*., 2002), reduced germination arising from toxic soil conditions (Anolifo *et al*., 2001), low water uptake and reduced nutrient availability (Merkl *et al*., 2005a), osmotic stress and root gas exchange (Ko and Day, 2004; Merkl *et al*., 2005b; Robertson *et al*., 2007), poor soil aeration and porosity (Kayode *et al*., 2009b) to soil quality in terms of fertility (Neumann and Martinoi, 2002; Essien and John, 2010). Species with high germination success and subsequent tolerance and growth in oil-contaminated soil conditions are more suitable for phytoremediation as opined by Oyedeji *et al*. (2014) and Oyedeji *et al*. (2015c).

Adverse effects of oil contamination on plants have been reported (Isirimah *et al*., 1989; Kulakow *et al*., 2000; Tesar *et al*., 2002; Robson *et al*., 2003; Merkl *et al*., 2004b; Kayode and Oyedeji, 2012). At high concentrations of oil in soil, most species suffer remarkably decreased growth rates (Amakiri and Onofeghara, 1983; Anoliefo and Okoloko, 2000; Kayode *et al*., 2009a). Agronomic parameters were

significantly different in the LTS when grown in crude oil-contaminated soils. However, some of the many selected LTS were able to tolerate the soil conditions and their growth was progressive during the study period, but growth (in terms of growth parameters stated above) of the LTS decreased with increased oil in soil. However, some LTS seedlings showed adequate tolerance in crude oil-contaminated soils. The growth parameters investigated are discussed under the following sub-sections.

6.2.1. Seedling height

High oil concentrations in soil decreased seedlings height and this was in accord with Anoliefo and Okoloko (2000), Kayode *et al*., (2009a), Bamidele and Igiri (2011) and Liao *et al*., (2015). Experimental results showed that seedling height of all species were significantly different at the various levels of contamination from those grown in non-contaminated soil. Correlation between seedling heights and contamination shows that the heights of *D. regia* were strongly negatively correlated (R^2 = -0.885; n = 25; P <0.01) (**Appendix 3.31**) and this attests to its good germination reported earlier. Similarly, correlation between seedling height and contamination also shows that the heights of *A. odoratissima* was the most strongly negatively correlated (R^2 = -0.930; n = 25; P <0.01) among the LTS. However, *P. pterocarpum* and *A. adianthifolia* were also strongly correlated (R^2 = -0.792; n = 25; P <0.01) and (R^2 = -0.911; n = 25; P <0.01), respectively (**Appendix 4.28**). Results showed that *D. regia* was associated with the greatest plant height and may best be suitable for phytoremediation.

6.2.2. Seedling girth

Oil contamination stunts plants growth. The girth of *D. regia* were strongly correlated (R^2 = -0.898; n = 25; P <0.01) (**Appendix 3.32**) with the girth of the two other species. *A. odoratissima* tended to produce the most significant result in terms of girth production. However, the correlation results of *P. pterocarpum* and *A. adianthifolia* were also significant (P <0.01) (**Appendix 4.29)**. These data support the earlier assertion of Amakiri and Onofeghara (1983) and Liao *et al*., (2015) that the presence of oil in soil at high concentration decreases plant growth and stem girth.

6.2.3. Leaf production

The presence of crude oil pollution decreased the number of leaves produced by the LTS seedlings. Seedlings grown on non-contaminated soils produced significantly more leaves than those grown in oil-contaminated soils. Leaf production was strongly correlated with contamination (R^2 = -0.898; n = 25; P <0.01) for *D. regia*, when compared with *B. monandra* and *T. tetratera* (**Chapter 3, Appendix 3.33**). Similarly, leaf production (**Chapter 4, Appendix 4.30**) showed that *A. odoratissima* tended to produce the most significant results in terms of leaf production. However, *P. pterocarpum* and *A. adianthifolia* were also strongly correlated at (P <0.01). The number of leaves in all species decreased as the concentration of crude oil in soil samples increased (**Sections 3.2** and **4.2**). This accords with Ezeala (1987), who observed effects of crude oil pollution on leaf production in *Pistia stratoites*. However, in this study, *D. regia* and *P. pterocarpum* had more leaf production over other LTS at the end of the study, thus, further suggesting their potential to tolerate in crude oil-contaminated soil and further enhanced their suitability for

phytoremediation and re-vegetation of crude oil-contaminated soil. *D. regia* and *P. pterocarpum* produced more seedlings and vegetative cover when grown on contaminated soil than any other LTS and this suggests them as good candidates for phytoremediation.

6.3. Nodule production

Nodulation ability of legumes is one of the crucial factors to consider in assessing the potential of LTS for remediation of crude oil-contaminated soil. Nodules habour microbial communities that can degrade contaminants through their root-soil relationships. Hence, nodule production plays a significant role in hydrocarbon degradation and replenishment of soil nutritional status. The number of nodules produced by LTS grown in crude oil-contaminated soils in the greenhouse varied, with *D. regia* and *P. pterocarpum* producing significantly more nodules than the other LTS. *T. tetraptera* had considerably lower nodule production as compared with all other LTS in the 100 ml crude oil-contaminated soil perhaps the microbes found the soil conditions too harsh to survive and in all the LTS, number of nodules decreased as crude oil concentration in soil increased. This may indicate that soil microbes have tendencies to survive better in low crude oil-contaminated soil than highly contaminated soil. Microbial populations in the root nodules play significant roles in TPH removal from soil systems, through rhizodegradation in the rhizosphere (Miya and Firestone, 2000; Kirk *et al*., 2005; Merkl *et al*., 2006).

In this study, rhizo-remediation/rhizodegradation of hydrocarbon compounds focused on the assessment of performance of the individual species as compared to non-planted soil for hydrocarbon removal/degradation. TPH degradation in the LTS

planted soils was significantly greater than the non-planted oil-contaminated soils. This may be due to microbial activities in the rhizosphere. Microbial population of hydrocarbon degraders is consistently greater in rhizosphere soil than soil without vegetation (Kuiper *et al*., 2004). Plant roots and their exudates increase microbial densities in the rhizosphere by one to four orders of magnitude, thus increasing microbial activities (Olson *et al*., 2003; Pilon-Smits, 2005). These increased microbial populations and activity give rise to rhizospheric effects in contaminated soil. Nodule production was significantly higher in *D. regia* and *P. pterocarpum* and the high prevalence of nodules may have exerted some influence on TPH degradation in their respective rhizosphere. Similar assertions were reported by Hutchinson *et al*. (2003) and Kuiper *et al*. (2004).

6.4. Plant biomass production

The ability to increase root and shoot biomass production is important in the determination of plant suitability for phytoremediation. The greatest removal of petroleum hydrocarbon concentration occurs during root production and plant species growth (Banks *et al*., 2003). However, no single trait can completely depict plant performance and thus a multi-factorial assessment approach, such as the one adopted in this study is recommended. The biomass production decreased with increased oil-contamination. This agrees with the findings of Merkl *et al*. (2004b), Barrutia *et al*. (2011) and Sharonova and Breus, (2012). Results of the current investigation provided evidence that *D. regia* and *P. pterocarpum* (and to a lesser extent *B. monandra*) are suitable species for phytoremediation, having exhibited more biomass production in the presence of crude oil-contamination than the other species (**Sections 3.4** and **4.4**). This suggests that these species may be capable of

switching their carbon allocation to roots under contamination and stressful soil conditions (Robson *et al*., 2003).

Plant root depth is important, since it is generally accepted that the physical and biological conditions favouring degradation of organic contaminants decreases with increasing soil depth (Olson *et al*., 2001). Some legumes have the capability to germinate in crude oil-contaminated soil and can combine high seedling emergence with high biomass production (Merkl *et al*., 2004b). However, the presence of petroleum hydrocarbons can significantly decrease plant biomass (Kulakow *et al*., 2000; Tesar *et al*., 2002; Robson *et al*., 2003). A good plant species for phytoremediation should produce an extensive root growth and biomass (Merkl *et al*., 2005a). Kulakow *et al*. (2000) investigated plant growth and biomass production of selected plant species in petroleum hydrocarbon contaminated soil in the USA for 180 days. They found that species were affected by the oil-contamination to varying degrees and there was considerable decrease in species growth and biomass production in contaminated soil compared to controls. Robson *et al*. (2003) made similar observation on grass and legumes native to Canada grown in hydrocarbon contaminated soils. *D. regia* and P. *pterocarpum* seedlings appeared less affected by oil-contamination and produced significantly higher roots and shoot biomass as compared to other LTS tested. This further suggests their suitability for phytoremediation and re-vegetation of crude oil contaminated soil.

6.5. Physicochemical properties of crude oil-contaminated soil planted with LTS

Soil has been described as non-renewable and dynamic ecosystem (FAO, 2005). The process of soil formation from rocks involves series of tremendously slow physicochemical and biological processes which ensure particle cleavage and aggregation through the activities of microbial populations (Banwart, 2011). Soil health has considerable impacts on plant metabolic processes, microbial density and diversity and has significant influences on human socio-economic life. Soil quality and viability to support plant yield and productivity depend extensively on the interaction between soil physicochemical and biological properties (Dexter, 2004). Soil properties can be significantly altered by crude oil-contamination and the soil in turn, can influence TPH degradation greatly (Germida *et al*., 2002). The characteristics of hydrocarbons present in soil contaminants (such as crude oil) determine their degradation and, to a lesser extent, soil properties are influenced (Aichberger *et al*., 2005). However, some soil properties including soil texture, SOM, pH and micro-nutrients affect hydrocarbon degradation (Chiapusio *et al*., 2007; Abii and Nwosu 2009).

6.5.1. Soil pH and electrical conductivity (EC)

Soil pH influences plant growth, ion solubility and availability, microbial activities and soil dispersion (Haynes and Naidu, 1998). The ideal soil pH range for optimal performance of microbial populations to degrade hydrocarbons depends largely on microbial species. In the current study, oil-contamination at different levels caused no significant variations in soil pH. Soil pH at all levels of oil-contamination tends to be acidic and the soil acidity was due primarily to the presence of crude oil in the

soil samples. However, introduction of LTS into the oil-contaminated soil significantly decreased soil acidity. This observation accords with Osuji and Adesiyan (2005), that crude oil-contamination increases soil acidity in the Niger Delta. The increased acidity in the oil-contaminated soil may be attributed to the oxidation of reduced sulphur compounds arising from the crude oil spilled into the soil environment. The degree of soil acidity and/or alkalinity is considered a major variable that affects nearly all soil properties, including physical, chemical and biological properties (Osuji and Adesiyan, 2005).

Introduction of LTS in the crude oil-contaminated soils slightly decreased soil acidity and this may have in turn played a vital role in the growth of tree species tolerant to oil-contamination. Soil pH results indicated that the presence of varying oil concentrations altered the soil pH considerably. In soil samples with high concentrations of oil, the soil pH tended to be more acidic. However, growing the LTS on such soil offered a considerable effect on pH at 16 WAP. For instance, *D. regia* improved soil pH from acidic to weakly acidic (5.30-5.70), *B. monadra* (5.28-6.03), *T. tetraptera* (5.51-5.83), *P. pterocarpum* (5.25-5.79), *A. adianthifolia* (5.55-6.34) and *A. odoratissima* (5.44-5.66) over the study period. The changes in pH of all crude oil-contaminated soil samples planted with LTS were not significantly difference (p <0.05) from the control at the end of the experiment. The change in soil pH noticed in the contaminated soils may be as a result of CO_2 evolving from the contaminated soil over time (Dalyan *et al*., 1990). However, decline in the impact of oil-contamination on agricultural soils is necessary for vegetation development. Although a significant elevation of soil pH was not achieved when LTS were grown on the crude oil-contaminated soil, a slight decrease in soil acidity

was achieved and these could be attributed to the growth of LTS on the contaminated soil. Increasing the period of growth of LTS on crude oil-contaminated soil may give rise to a significant elevation of soil pH.

The site soil had higher Soil Electrical Conductivity (EC) than the various crude oil-contaminated soils. The presence of crude oil in the soil sample may have lowered soil EC. However, improvements in soil EC in the crude oil-contaminated soils may suggest effectiveness of remediation processes, particularly phytoremediation to improve EC in soil. Similar observations were made by Tanee and Albert (2011), who investigated post-remediation assessment of crude oil polluted site at Kegbara-Dere community, Rivers State, Nigeria.

6.5.2. Soil organic matter (SOM) and soil organic carbon (SOC)

SOM and SOC are co-indices of soil fertility and plant productivity. Often, SOM originates from natural materials, including plant and animal litter and microbial biomass. SOM may also originate from artificially-produced chemicals, including pesticides, hydrocarbons, plastics and industrial effluent (FAO, 2005). SOM influences nutrient bioavailability and soil enzyme production by microbial populations, including bacteria and fungi in association with root systems (Chaudhary *et al*., 2012). SOM affects the transportation and bioavailability of petroleum hydrocarbons and there are close relationships between SOM and petroleum hydrocarbons in soil (Chaudhary *et al*., 2012). There were significant increases in SOM after oil-contamination of soils (Liu *et al*., 2007). Similar trends were observed in the current study for SOM and SOC. SOC and SOM were observed to be 2.48 and 4.28%, respectively, in the site soil. SOM is capable of supporting the growth of plant species as most surface soil within the depth of 15 cm in the study

area has SOM in the range of 0.1-7.9% (Ayodele and Omotoso, 2008). Cumulatively, the slightly higher values of % SOM in the current study (**Sections 3.5** and **4.5**), relative to the control experiment gives evidence of soil contamination with crude oil and affirms a possible relationship between SOM and oil-contamination. This work also agrees with Osuji and Adesiyan (2005) who proposed that SOM and SOC contents of soil increased due to artificial crude oil contamination. However, the most plausible explanation for the slight increase in SOM and SOC contents may be the carbon supplement from the hydrocarbons in the crude oil and potential of the crude oil to alter metabolic processes, which would have facilitated mineralization.

SOM and SOC values have far reaching implications for mineralization, because carbon mineralizing capacity is directly related to the SOC content of soil. This usually decreases oxygenation, which in turn, affects microbial metabolism. CO_2 is released from completely anaerobic systems through the activity of anaerobes, and all these increase stresses on living organisms in soil. The increase in SOM in the LTS planted soils among the crude oil-treated soils shows that the LTS have metabolic and absorption capabilities and transport systems that selectively removed contaminants from the growth matrix. Alteration in the SOM and SOC in the current study agrees with Thoma *et al*. (2002), who observed a similar trend of SOM and SOC in a soil sample contaminated with 3% by weight weathered crude oil that was phytoremediated with *Aeschynomene americana* (also a leguminous plant species).

6.5.3. Soil macronutrients (N, P and K)

Macronutrients including N, P, K, Na, Ca and Mg are essentially required for normal growth of plant species and nutrient results were reported in **Sections 3.5** and **4.5**. N and P are usually limiting in crude oil-contaminated soil (Germida *et al*., 2002; Adam and Duncan, 2002; Hutchinson *et al*., 2003) and similar observations were made in this study. However, a slight change in the values of these nutrients was noticed when planted with LTS. Similar trends were observed in K. Results of selected macronutrients indicated that N, P and K were altered in the LTS planted soil as compared to controls, but there was no significant *(p* <0.05) difference. The observed increases were higher in crude oil-contaminated soils planted with *D. regia* and *P. pterocarpum*. The soils planted with *T. tetraptera* and and *A. adianthifolia* had decreased N and P. These show that the LTS were effective in maintaining the soil N and available P balance to such a level that bioaccumulation compensated for any decrease in N and P.

The LTS have mechanisms in their root systems that provide root exudates (energy, nutrients and enzymes) to microbial populations in the rhizosphere (Cunningham *et al*., 1996; Grayston *et al*., 1996). These exudates induce, or enhance, microbial populations, which result in enhanced degradation of organic contaminants in the rhizosphere. The added N to the contaminated soil by the LTS root systems could be used by the microbes engaged in degradation processes in the rhizosphere. Plants also influence nutrient cycling and supply in the soil. Organic substrates from root systems are one of the vital factors that influence nutrient availability in the rhizosphere (Grayston *et al*., 1996). Some organic compounds in root exudates, such as phenolics, organic acids, proteins and alcohols, are potential sources of C and N

for microbial populations, which are capable of degrading hydrocarbon compounds in the soil (Alkorta and Garbisu, 2001). The chemical composition of root exudates and rates of exudation differ considerably in plant species and between different stages of plant development (Germida *et al*., 2002).

Petroleum hydrocarbon contamination modifies C:N ratio in crude oil-contaminated soils (Adam and Duncan, 2003). The authors postulated that additional C from hydrocarbon compounds could stimulate microbial populations, but can also result in an imbalance in the C:N ratio, which can cause soil N immobilization and N-deficiency for plant growth and probable remediation potential. However, the presence of LTS grown on the crude oil-contaminated soils in the present study catered for these shortcomings. The observed increases in the nutritional status may be due to N-fixation in the root nodules of LTS and the decay of leaf litter from seedlings onto the soil. LTS are capable of fixing atmospheric N and harnessing other potential sources within the root systems to correct N-deficiency in the crude oil-contaminated soils. Thus, the LTS and their associated microbial populations were able to grow well and combat TPH degradation in oil-contaminated soils over 16 weeks. This positive effect may be due to the contribution of N-fixation by legumes and their microbial symbiotic relationships.

N and P are important inorganic nutrients that are most often limited in crude oil-contaminated soils (Germida *et al*., 2002; Hutchinson *et al*., 2003) and these affect phytoremediation processes. Fertilization of crude oil-contaminated soils by augmentation may enhance remediation of such oil-contaminated soil (Dakora and Philips, 2002; Merkl *et al*., 2005c). However, not all phytoremediation processes are

considerably enhanced by nutrient augmentation (Kirkpatrick *et al*., 2006). Rentz *et al*., (2003) reported decreased microbial degradation activities after addition of N and P amendments, and attributed the decline in microbial activity to inhibition of oligotrophic degraders or the stimulation of non-competent bacteria (Olson *et al*., 2003). Some of the LTS grown in this study portrayed some adaptive features, including N-fixation ability and recorded strong growth and development on the crude oil-contaminated soil without fertilizer application. This suggests that the addition of fertilizers sometimes practised in phytoremediation processes may not be necessary and can pose harmful effects on natural vegetation. Since fertilizers were not added in this study, a 'worse-case scenario' of crude oil-contamination in soil and subsequent remediation is presented. Some phytoremediation research has investigated the use of other plant species for remediation with or without fertilization and a great deal of effectiveness has been demonstrated for TPH removal and soil recovery under both fertilizer and non-fertilizer amended conditions (Merkl *et al*., 2005c; White *et al*., 2003, 2006; Brandt *et al*., 2006; Tanee and Kinako, 2008; Chorom *et al*., 2010).

6.5.4. Exchangeable ions (Na, Ca and Mg)

The results of the soil analyses show that LTS were effective in altering the concentrations of exchangeable ions in the crude oil-contaminated soils. These soil nutrients are transferred within the rhizosphere as ions. Crude oil contamination changes concentrations of the exchangeable ions, such as Na^{+}, Ca^{2+} and Mg^{2+}. The amount of all exchangeable ions in LTS planted soils significantly increased (p <0.05) over the duration of the experiment. This may imply that the root systems of LTS had the capability of enhancing the distribution of exchangeable ions within the

soil compartments. Crude oil contamination is capable of introducing additional exchangeable ions, including Na^+, to soil. Abii and Nwosu (2009) investigated the impact of crude oil spill in polluted and unpolluted areas of River State, Nigeria, and reported high Na^+ concentrations in crude oil-contaminated areas. Similar trends were observed by Onyeike *et al.* (2000), who reported such increases in exchangeable ions of soils from crude oil-polluted soil in Ogoni land. The increased Ca^{2+} and Mg^{2+} observed in both the control and contaminated soils may be due to oil-contamination. Soil nutrient antagonism resulting in nutrient imbalances may have accounted for changes in some soil properties (Ogboghodo *et al*., 2005), but the root systems of the LTS distributed the ions within the soil and decreased nutrient imbalances. These nutrients in turn, contributed to the growth of the LTS and Ca^{2+} ions may have provided the LTS with adequate tensile strength against wind blows, which could cause shoot breakage during growth.

The presence of crude oil in soil may have altered some soil physical properties, including soil bulk density, soil aeration and porosity, as the crude oil-contaminated soils became more sticky and compacted. Ewetola (2013) and Kayode *et al.* (2009b) made similar observations that oil-contamination could block soil pore spaces and consequently impair soil aeration, porosity and water infiltration, which have negative effects on plant growth. However, the extensive root systems of the LTS in the study changed the crude oil-contaminated soil and may further suggest their potential usefulness in phytoremediation of crude oil-contaminated soils.

6.6. Microbial counts in the crude oil-contaminated soil planted with LTS

Soil biota exist in three main categories, including soil flora, soil fauna and soil micro-organisms (microbes), which are greatly inter-related in their activities (McCauley, 2005). Soil fauna operate as conditioners in decomposing organic debris and promoting soil nutrient cycling and therefore, soil fauna can be pivotal in bioremediation processes (Yin *et al*., 2010). Microbial activity is one of the main factors influencing petroleum hydrocarbon degradation in the rhizosphere (John *et al*., 2011; Chen *et al*., 2013). These microbes are capable of breaking down complex hydrocarbons to simple and harmless compounds. Microbial counts of complex petroleum hydrocarbon-degrading microbes have been reported to be higher in the rhizosphere soil than non-vegetated soil (Kupier *et al*., 2004; Johnson *et al*., 2005; Joner *et al*., 2006). Plant root systems and their exudates potentially aid microbial breeding and activity (Olson *et al*., 2003; Pilon-Smiths, 2005; Gaskin and Bentham, 2005; Kirk *et al*., 2005; Joner *et al*., 2006; Mueller and Shann, 2006). The success of phytoremediation on crude oil-contaminated soil does not rely on the germination and tolerance of plants species alone, but also on plant-microbe specific relationships for the degradation of contaminants. Soil biological activities are crucial for reclamation and restoration of crude oil-contaminated soil and also provide useful information by serving as inexpensive bio-indicators for contaminated soil (Maila and Cloete, 2005; Riffaldi *et al*., 2006).

The results obtained in the current study show that there were increased microbial counts in the crude oil-contaminated soil planted with LTS. All the LTS stimulated microbial populations in the crude oil-contaminated soils than non-LTS planted soils. The presence of hydrocarbons can stimulate microbial growth and populations

(Gaskin and Bentham, 2005), but vegetation potentially provides additional influences. Vegetation growing on oil-contaminated soil can increase microbial counts in the rhizosphere (Kirk *et al*., 2005) and in turn the microbial populations degrade contaminants and enhance plant growth (Chen *et al*., 2013). Soil microbes are capable of adjusting to environmental changes and the organisms that can survive the modified conditions tend to have the highest populations (Cappello *et al*., 2007). Contamination of soil with hydrocarbons might increase hydrocarbon degrading micro-organisms in soils (Vidali 2001; Kim *et al*, 2006). This was demonstrated in the current study.

The microbial populations in *D. regia*, *B. monandra*, *P. pterocarpum* and *A. odoratissima* increased in the crude oil contaminated soil at low (25 ml) and moderate (50 ml) contamination more than in the non-planted contaminated soils after 16 weeks growth period. This may have subsequently produced positive effects on hydrocarbon degradation in the crude oil-contaminated soils. Microbes, whose growth was influenced by the LTS, may have broken down the complex hydrocarbons for their respiration and nutrition processes. This suggests that the presence of plants provided additional influences on soil microbial counts and subsequently, are capable of hydrocarbon degradation. It appears that LTS root systems provide microbial populations with an array of conditions that help to selectively sustain their growth in crude oil-contaminated soil. The presence of vegetation may have influenced many physical conditions in the soil, including structure, porosity, infiltration and aeration (Hutchinson *et al*., 2003) and these soil properties have tendencies to influence microbial activity through regulation of water and nutrient transportation. Plant root systems can contribute amino acids,

carbohydrates, growth factors and soluble proteins (Miya and Firestone, 2000; Lee *et al*., 2008).

Crude oil-contaminated soils are generally deficient in N (Wenzel, 2009), but the root exudates of the LTS root systems may have been another source of N for microbial populations (in addition to the N-fixation ability of the LTS) and in turn counter-act N deficiency in crude oil-contaminated soil. Although stimulation of hydrocarbon degrading microbes was noted in the LTS, the degree of influence on microbial density between species was different. Specific-species relationships with oil-contaminated soils were noted, and a comparative estimate shows that soil planted with *D. regia* and *P. pterocarpum* had the highest influence on the promotion of hydrocarbon degrading microbial populations in the rhizosphere. Hydrocarbon degrading microbial populations are selectively enriched in the rhizosphere, as demonstrated in the selected LTS. Thus, the current study agrees with earlier reports that microbial populations are abundant in the rhizosphere (Miya and Firestone, 2000; Kirk *et al*., 2005; Merkl *et al*., 2006).

Kim *et al*. (2006) reported increased microbial community composition in the rhizosphere of *Medicago sativa* (Fabaceae) grown on diesel-contaminated soil for phytoremediation. The study showed that the combined effect of diesel-contamination and plant roots, strongly influenced microbial growth and populations. They also found that total microbial activity and prevalence of hydrocarbon degrading micro-organisms was highest in the diesel-contaminated rhizosphere soil as compared with non-planted soil. It was noted that 82% and 59% of hydrocarbon removal were achieved in soils planted with *Medicago sativa* and

non-planted soil, respectively, after seven weeks. Similarly, Miya and Firestone (2000) reported that the rhizosphere of *Avena bartata* (Poaceae) selectively enriched the phenanthrene degrading microbial populations by as much as one order of magnitude as compared to non-planted soil. Furthermore, Merkl *et al*. (2006) reported that *Brachiaria brizantha* influenced microbial populations and hydrocarbon degradation in oil-contaminated soil. The authors observed that the presence of the plant species produced significant increases in microbial growth and populations capable of degrading alkanes, but did not achieve significant increments of aromatic and cycloalkane degrading microbial populations compared with non-planted soil. Furthermore, Kirk *et al*. (2005) affirmed an increased number of diesel degrading bacteria in the *Lolium perenne* (Poaceae) rhizosphere after a period of seven weeks as compared to non-planted soil. There was comparable hydrocarbon degrading population increases in the current study involving LTS.

All LTS supported more than one order of magnitude higher numbers of hydrocarbon degrading microbial populations in the rhizosphere relative to the non-planted soil. Temperature is an important environmental factor, which plays a crucial role in microbial growth (Kirk *et al*., 2005). The greenhouse temperature was 37.0-38.5ºC during experiments and this may have accounted for the more rapid microbial growth in the LTS rhizosphere. Brandt *et al*. (2006) opined that phytoremediation processes are effective at temperature ranges of ~37º-40ºC. The LTS assessed in the current study influenced microbial growth and populations, which enhanced the plant-induced changes in soil microbial density and activities. These changes were species-specific and enhanced rhizodegradation (biodegradation) of complex petroleum hydrocarbons in crude oil-contaminated soils.

6.7. Hydrocarbon degradation and removal in the rhizosphere of LTS and non-planted crude oil-contaminated soil

The ability of indigenous soil microbial populations for petroleum hydrocarbon-contaminated soil remediation is well known (Frick *et al*., 1999; Reynolds *et al*., 1999; Pichtel and Liskanen, 2001; Jorgensen, 2007). The process is sometimes slow and with restricted outcomes, owing largely to limited substrate availability and other inhibitory parameters (Tanee and Kinako, 2008). Environmentally-friendly technologies are required to stimulate natural decomposition processes in oil-contaminated soils, thus reducing hazards to public health, ecosystem disturbance and rehabilitation of oil-contaminated soils. The results of the current study on LTS hold enormous potential for microbial population stimulation and enhancement in the rhizosphere (as shown in **Chapter 3** and **4** and in the literature review) and this could have high impacts on hydrocarbon degradation in crude oil-contaminated soils. The ability of LTS to enhance hydrocarbon degradation principally by providing an optimum environment for microbial proliferation in the rhizosphere observed in the current study agrees with Adam and Duncan (1999). Vegetation influences hydrocarbon degradation through root exudation of organic compounds that stimulate the activity of microbial populations in the rhizosphere, thus increasing biodegradation rates (Hutchinson *et al*., 2003; Kupier *et al*., 2004; Kaimi, 2006).

The results of this study show the extent of hydrocarbon removal from crude oil-contaminated soil planted with LTS. Results also showed that hydrocarbon removal was significantly higher in LTS planted soil than in non-planted soil and the findings accord with Zand *et al*. (2010) and Liao *et al*. (2015). Among all the LTS investigated, *D. regia* planted soils recorded the greatest hydrocarbon removal

efficacy (180.19-188.82 mg/kg), followed by *P. pterocarpum* (174.27-187.56 mg/kg) and there was significant difference in hydrocarbon removal in the two species. However, the rate of hydrocarbon removal in the other tree species rhizosphere was considerably lower than observed in *D. regia* and *P. pterocarpum.* These two tree species facilitated greater removal of TPH as compared to non-planted soil (116.66-142.54 mg/kg). Evidence of rhizosphere degradation through microbial population stimulation by the presence of oil and root systems was demonstrated for all the LTS. However, there were variations in TPH removal among the various oil treatments grown with plants. The extent of rhizosphere degradation by the species differs considerably and this suggests there were species-specific degradation differences among the LTS.

The contribution of plant species to hydrocarbon contaminant removal from soil could be argued in several ways. Firstly, the direct interaction between root systems of seedlings and hydrocarbon contaminants in the soil through sorption processes through diffusion, root uptake mechanisms and transportation via the root systems to aerial parts (Gunther *et al.*, 1996). Uptake and transport of crude oil hydrocarbons into the root and shoot systems were minimal when the selected LTS were grown on crude oil-contaminated soil. Secondly, the microbial population was possibly stimulated, largely due to interactions in the rhizosphere causing hydrocarbon degradation (Glick, 2003). This is likely to play a vital role in influencing the fate of hydrocarbon degradation in crude oil-contaminated soil. Microbial species are capable of digesting hydrocarbons and utilizing the resulting compounds as food and energy sources for growth and reproduction (Wang *et al*., 2011). Simultaneously, the hydrocarbons are hydrolyzed from complex organic compounds to simple and non-

toxic compounds, such as CO_2 and H_2O, along with microbial accumulation through oxidation processes of aerobic respiration. Some soil anaerobic microbial populations are capable of degrading hydrocarbons through reduction processes. Thirdly, environmental conditions and soil properties may have influenced the growth of the selected LTS, which in turn may have increased hydrocarbon degradation in the rhizosphere compared with non-planted soils (Germida *et al*., 2002).

Relevant environmental factors including, oxygen and soil nutrients are important in hydrocarbon degradation. Oxygen plays a vital role in hydrocarbon phytoremediation, most especially the aliphatic hydrocarbons (Olson *et al*., 2003; Bamforth and Sinleton, 2005). Plants have the capability to enhance oxygenation in oil-contaminated soils and thus improve remediation processes. Plant root systems operate as pathways for transportation of oxygen to the root zone and thus enhance aerobic respiration conditions for phytoremediation. Root systems also increase soil porosity and enhance diffusion of atmospheric O_2 (Rentz *et al*., 2003). Plant root systems have the ability to increase soil moisture content by promoting an effective pathway for water movement (Jing *et al*., 2008) and the moisture content in turn provides the microbial populations with their optimum moisture that favours hydrocarbon degradation. LTS influenced rhizospheric hydrocarbon degradation and TPH removal in crude oil-contaminated soil in the current study. The levels of hydrocarbons observed in the planted soils show that the LTS were very efficient in their rhizosphere degradation of crude oil hydrocarbon constituents.

6.8. Establishment of LTS in the field

The selected LTS were able to grow in the field, but *D. regia* produced significantly more growth in terms of plant height, girth and leaf production (**Section 3.5.5**) as compared with all other LTS. However, a direct *in situ* experimentation of the feasibility of its growth on crude oil-contaminated site will prove its field suitability and growth success. Ogunika and Kayode (2005) reported the significance of LTS for N-fixation in tropical N-deficient soils. N-fixation *in situ* and leaf fall addition are crucial processes by which LTS contribute to increased soil fertility. LTS are capable of producing large biomass, which can release nutrients and increase soil fertility. These LTS have assumed special importance in agroforestry systems as a viable N source, which produces significant soil amelioration (Ogunika and Kayode, 2005; Mubiru and Coyne, 2009) and their vegetative cover have been found in the current study and the literatures to be very important for contaminated soil remediation in tropical soils.

6.9. Oil sorption by tropical kaolinite and its re-usability for oil spill clean up

Zeolites are stable three-dimensional crystalline, micro-porous materials, with many potential applications (Metes *et al*., 2004). Their properties make them potentially useful for many industrial purposes. Zeolite minerals have proved useful in such processes as catalysis, molecular sieving, refining, ion exchange and environmental protection and management (Chiang and Chao, 2001; Bebon *et al*., 2002; Caballero *et al*., 2007; Xu *et al*., 2007; Ajayi *et al*., 2012). They have also proved useful in many processes including filtration process, odour control, water softening and adsorption processes, soil stabilization and conditioners, soil amendments, slow-

release fertilizers, soil-less substrates, carriers for insecticides and pesticides, water treatment, paint components with anti-corrosive properties, fixation of phosphates, clean-up of sewage, ammonium ion removal and as remediation agents in contaminated soils (Ming and Allen, 2001; Polat *et al.*, 2004; Terzano *et al*., 2005; Jakkula *et al*., 2006).

Clays and natural zeolites such as clinoptilolite (Obua *et al*., 2014), Na-chabazite (Kelay *et al*., 2015) and kaolinite (Oyedeji *et al*., 2015a) have been investigated as partial solutions to environmental contamination with oil spills. These natural zeolites are applicable to environmental contamination remediation, owing largely to their inherent properties, such as uniform pore size/shape, hydrophilicity/hydrophobicity, plasticity and high sorption capacity (Berendsen *et al*., 2006). Their physicochemical properties also make them suitable for agricultural uses (Barbarick and Pirela, 1984; Allen and Ming, 1993, Leggo, 2000; Jakkula *et al*., 2006; Leggo *et al*., 2006) and as soil remediating agents for environmental protection (Ming and Allen, 2001; Polat *et al.,* 2004; Terzano *et al*., 2005).

The current study has shown the efficacy of kaolinite (10 and 20 g samples) applications as suitable and effective sorbent agents for oil-contamination at different levels. Samples of 10 g kaolinite sorbed a mean of 75.28, 74.68, 61.52 and 60.80% of the 25, 50, 75 and 100 ml of Automobile Oil (AO) treatments, respectively. The sorption potential of kaolinite increased with the increase in kaolinite to 20 g. Samples of 20 g kaolinite had mean sorption of 93.44, 87.04, 73.49 and 70.86% for 25, 50, 75 and 100 ml AO treatments, respectively. Thus, oil sorption increased as the amount of oil contamination decreased (**Table 5.2**). However, sorption of the

various oil-contamination levels was unique, but the 25 and 50 ml oil-contaminations representing low and moderate contaminations, were most strongly sorbed. Statistical analysis also showed that sorption was significantly different among the oil treatments ($P < 0.05$) (**Appendix 5.1** and **5.4**).

The rates of kaolinite application on oil contamination sorbed large proportions of oil contamination. AO contamination and sorption rate in the 10 and 20 g samples were strongly positively correlated ($R^2 = 0.975$; $n = 5$; $P < 0.05$) (**Figure 5.2, Appendix 5.7**). This implies that the volume of oil sorption is directly proportional to the amount of oil contamination, but inversely proportional to percentage sorption. The potential re-usability of kaolinite after the initial use for oil sorption was analysed and it was observed that the kaolinite was re-usable. Kaolinite was still effective for oil-sorption, although, the rate of sorption tended to be less than fresh kaolinite. The 10 g burnt kaolinite sorbed 43.62% while 20 g sorbed 58.90% of 100 ml of oil. The initial sorption experiment revealed sorption rates of 10 and 20 g fresh kaolinite for 100 ml oil contamination as 60.80 and 70.86%, respectively (**Table 5.4**). The results suggest that the rate of oil sorption by fresh kaolinite is higher than burnt kaolinite. Some of the kaolinite pore spaces may have been blocked during the first usage. However, the burnt kaolinite could be potentially re-used for oil sorption. These observations accord with others (Obua *et al*., 2014; Kelay *et al*., 2015), who investigated the effectiveness and re-usability of clinoptilolite and Na-chabizites respectively, for oil-sorption.

Zeolites are used in agriculture as soil amendments and some of the relevant qualities include: decreased soil acidity; activating nutrients from soil reserves and

decreasing the need for mineral fertilizers, thus eliminating fertilizer acidifying effects; toxic effects and increasing drought resistance by binding water molecules (Pisarovic *et al*., 2003). The role of natural zeolites, such as clinoptilolite, in agriculture encourages plants growth (Leggo, 2000; Manolov *et al.*, 2005; Leggo, *et al.*, 2006; Azarpour *et al*., 2011; Ghiasi and Jasour, 2012) and soil system improvement through use as soil-amendments (Manolov *et al*., 2005; Adbi, *et al*., 2006; Földesová *et al.*, 2007). The high cation-exchange capacities, cation selectivity and molecular sieving abilities make them suitable for contaminated soil remediation (Ming and Allen, 2001).

Some zeolites, fertilizers, sawdust and manure have been utilized in remediation processes. Application of fertilizers proved useful in bioremediation processes (Merkl *et al*., 2005c; Brandt *et al*., 2006; Tanee and Kinako, 2008; Chorom *et al.*, 2010), but some negative effects place limitations on its use in phytoremediation (Pisarovic *et al*., 2003). The efficiency of natural zeolites, such as clinoptilolite, as an effective absorbents of petroleum products and soil amendments (Misaelides, 2011; Obua *et al*., 2014), Na-chabazite (Kelay *et al*., 2015), kaolinite (Oyedeji *et al*., 2015a) has been demonstrated, with minimal or no damage to plants and soils. The importance of kaolinite for environmental remediation and oil-spill sorption and clean up has been emphasized in this study.

Nigeria has abundant kaolinite clay resources (**Figure 1.9**), which occur in many parts of the country (Kovo, 2011; Oyedeji *et al*., 2015a), but available clays in the different deposits have a great deal of differences and frequencies (Fakolujo *et al*., 2012). Notable among the Nigerian kaolinite deposits are Kankara in Kastina State

(Atta *et al*., 2007; Ajayi *et al*., 2012), Ahoko in Kogi State (Kovo, 2011), Ukpor in Anambra State (Igbokwe *et al*., 2008), Ovwian in Delta State (Oghenejoboh and Ohimor, 2011), Ubulu-Ukwu in Delta State (Adebowale *et al*., 2005) and Abeokuta deposit in Ogun State (Fakolujo *et al*., 2012). Large kaolinite deposits occur in the Niger Delta region, a region with abundant crude oil resources and frequent oil spillages. Kaolinite has commendable potential to remediate contaminated environments and given their abundance in Nigeria, they could be used to pre-treat contaminated soil prior to phytoremediation. The current study, therefore, presents fresh and burnt kaolinite as potential sorbents of crude oil spill and possibly an enhancement for the growth of LTS planted on crude oil-contaminated soil for phytoremediation processes. It may therefore, serve as a suitable pre-treatment and soil amendment for oil spillage remediation in tropical soils generally and the Niger Delta of Nigeria in particular.

6.10. Overall Conclusions

The conclusions drawn from the current study may be summarized as follows:

1. Oil-contamination affects the germination and growth of Leguminous Tree Species (LTS) and the physicochemical properties of the soil. The effects can be decreased by planting and allowing the tolerant LTS to grow over a period of time.
2. *D. regia* and *P. pterocarpum* are tolerant and capable of influencing microbial populations in their rhizosphere for hydrocarbon degradation, thus decreasing the effect of oil-contamination on plant growth and soil.
3. *D. regia* and *P. pterocarpum* LTS are potentially suitable for phytoremediation and re-vegetation of crude oil-contaminated tropical soil. Timber produced from

such tolerant plant species could provide some economic gains and serve as a source of timber for local needs.

4. Kaolinite resources, which are widely available in the tropics, can be used as pre-treatment and amendment agents of oil-contamination before phytoremediation procedures with LTS on such oil-contaminated soil.
5. An effective and environmentally-friendly remediation option for crude-oil contaminated soils, which utilizes simple and inexpensive method of using 'nature to cleanse nature' through phytoremediation with native LTS and kaolinite pre-treatment has been proven and is recommended for use in tropical oil-producing nations, particularly Nigeria.

The investigation revealed the potential of LTS, particularly *D. regia* and *P. pterocarpum* (and to a lesser extent, *A. odoratisisima* and *B. monandra*) as suitable and tolerant plant species capable of growing in crude oil-contaminated soils of Niger Delta region of Nigeria. They enhanced hydrocarbon degradation in their rhizosphere, thus remediating the oil-contaminated soil over time. The tree species were sensitive to varying levels of oil-contamination. Therefore the tree species could also serve as bio-indicators of the effects of oil spillages in ecosystems. Artificial contamination of soil is preventable, but sometimes, it is unavoidable during oil exploration and therefore, the importance of remediating such contaminated soil with native tolerant plant species growing in such areas will enhance natural cleaning processes, through microbial activities in the rhizosphere. Earlier reports and the current study indicate that phytoremediation techniques are yielding positive outcomes and gaining considerable societal attention and acceptance. However, it has some limitations, including selection of suitable native

tolerant plant species. These limitations could only be overcome by further research on tolerant and suitable plant species for phytoremediation protocols.

Some of the LTS investigated in the current study have proven their suitability for remediation of crude oil-contaminated soils and can be exploited by individuals, oil prospecting companies and government agencies to remediate crude oil-contaminated soils. The ability of kaolinite resources to sorb oil-contamination also proved viable. It is, therefore, proposed that kaolinite resources be used as pre-treatment and amendment agents of oil-contamination before phytoremediation procedures are practised with LTS on such oil-contaminated soil. This information is crucial for developing plans for soil conservation, restoration, reclamation and sustainable management in tropical oil-producing countries generally and in the Niger Delta region of Nigeria in particular.

Suggestions for future research

The scope of this study could be expanded to cover other interesting areas and these can be summarized as follows:

1. What plant-specific exudates are present in the rhizosphere during the remediation process?
2. What is the effect of crude oil-contamination on plant root and shoot anatomy? Does oil-contamination alter the anatomical structures of the tree species?
3. What is the effect of oil-contamination on nutrient cycling within the rhizosphere? Where specifically do N, P and K come from when oil-contaminated soil is vegetated with plants?

4. Can nodule production be increased for enhanced remediation of crude oil-contaminated soil in LTS? What role can bioaugmentation with additional microbial isolates play in the remediation processes?
5. What phytoremediation outcomes could be achieved using the selected LTS on aged or weathered crude oil-contaminated soil and establish a comparison with freshly contaminated soils?
6. What comparisons could be drawn from the two best performing species for remediation processes of crude oil-contaminated soil over longer periods?
7. What comparisons could be established between LTS fertilized and non-fertilized phytoremediation protocols?
8. What is the long-term performance of the selected LTS in the Forest of Ayodele?
9. What is the effectiveness of the combination of bioaugumentation with soil microbes, kaolinite and LTS for crude oil-contaminated soil remediation?
10. What is the effectiveness of kaolinite pre-treatment and phytoremediation protocols using LTS on crude oil-contaminated soils? A practical field study on crude oil-contaminated soil in the Ogoniland area of Nigeria is proposed.

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APPENDICES

Appendix 2.1: Mathematical determination of crude oil concentration in the contaminated soils

Note: 1 ml equivalent to 1 cm^3.

For 0 ml crude oil-contaminated soil:

0 ml equ. 0 cm^3

0/4000 x 100%

= 0.00% v/w

For 25 ml crude oil-contaminated soil:

25 ml equ. 25 cm^3

25/4000 x 100%

=0.63% v/w.

For 50 ml crude oil-contaminated soil:

50 ml equ. 50 cm^3

50/4000 x 100%

= 1.25% v/w.

For 75 ml crude oil-contaminated soil:

75 ml equ. 75 cm^3

75/4000 x 100%

= 1.88% v/w.

For 100 ml crude oil-contaminated soil:

100 ml equ. 100 cm^3

100/4000 x 100%

= 2.50% v/w.

Therefore, the concentration of crude oil in the soils contaminated with 0, 25, 50, 75 and 100 ml crude oil are 0.00, 0.63, 1.25, 1.88 and 2.50% v/w, respectively.

Appendix 3.1: Analysis of Variance (ANOVA) of *D. regia* germination at different levels of contamination

Dependent Variable: Treatments

Source	Sum of Squares	df	Mean Square	F	P
Corrected Model	47.200[a]	8	5.900	8.741	<0.001
Intercept	1681.000	1	1681.000	2490.370	<0.001
Concentration	44.400	4	11.100	16.444	<0.001
Rep	2.800	4	0.700	1.037	0.419
Error	10.800	16	0.675		
Total	1739.000	25			
Corrected Total	58.000	24			

R Squared = 0.814 (Adjusted R Squared = 0.721).

Appendix 3.2: Correlation analysis between contamination and germination of leguminous species

Correlation of variables		Contamination	*T. tetraptera*	*B. monandra*	*D. regia*
Contamination	Pearson Correlation	1.0	-0.990**	-0.988**	-0.976**
	P (2-tailed)	<0.001	<0.001	0.002	0.004
	N	5	5	5	5
T. tetraptera	Pearson Correlation	-0.990**	1.0	0.989**	0.996**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	5	5	5	5
B. monandra	Pearson Correlation	-0.988**	0.989**	1.0	0.972**
	P (2-tailed)	0.002	<0.001	<0.001	0.006
	N	5	5	5	5
D. regia	Pearson Correlation	-0.976**	0.996**	0.972**	1.0
	P (2-tailed)	0.004	<0.001	0.006	
	N	5	5	5	5

**Correlation is significant at P <0.01 (2-tailed).

Appendix 3.3: ANOVA of *B. monandra* germination at different levels of contamination

Dependent Variable: Treatments

Source	Sum of Squares	df	Mean Square	F	P
Corrected Model	57.120[a]	8	7.140	9.333	<0.001
Intercept	1536.640	1	1536.640	2008.680	<0.001
Concentration	55.360	4	13.840	18.092	<0.001
Rep	1.760	4	.440	.575	0.685
Error	12.240	16	.765		
Total	1606.000	25			
Corrected Total	69.360	24			

R Squared = 0.824 (Adjusted R Squared = 0.735).

Appendix 3.4: ANOVA of *T. tetraptera* germination at different levels of contamination

Dependent Variable: Treatments

Source	Sum of Squares	df	Mean Square	F	P
Corrected Model	32.080[a]	8	4.010	5.854	<0.001
Intercept	1324.960	1	1324.960	1934.248	<0.001
Concentration	29.440	4	7.360	10.745	<0.001
Rep	2.640	4	0.660	0.964	.454
Error	10.960	16	0.685		
Total	1368.000	25			
Corrected Total	43.040	24			

a. R Squared = 0.745 (Adjusted R Squared = 0.618).

Appendix 3.5: ANOVA between mean germination of leguminous tree species and contamination

Dependent Variable: Mean of germination of plant species

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.312[a]	6	4.552	53.764	<0.001
Intercept	906.371	1	906.371	10705.165	<0.001
Concentration	25.163	4	6.291	74.299	<0.001
Species	2.149	2	1.075	12.693	0.003
Error	0.677	8	0.085		
Total	934.360	15			
Corrected Total	27.989	14			

A. R Squared = 0.976 (Adjusted R Squared = 0.958).

Appendix 3.6: Correlation analysis of co-efficient of velocity (CoV) of germination among leguminous tree species moistened with varying crude oil-contaminated soil water extracts

Correlation of variables		Contamination level	*D. regia*	*B. monandra*	*T. tetraptera*
Contamination level	Pearson Correlation	1.0	-0.879*	-0.992**	-0.819
	P (2-tailed)	<0.001	0.050	<0.001	0.090
	N	5	5	5	5
D. regia	Pearson Correlation	-0.879*	1.0	0.858	0.644
	P (2-tailed)	0.050	<0.001	0.063	0.241
	N	5	5	5	5
B. monandra	Pearson Correlation	-0.992**	0.858	1.0	0.885*
	P (2-tailed)	0.001	0.063	<0.001	0.046
	N	5	5	5	5
T. tetraptera	Pearson Correlation	-0.819	0.644	0.885*	1.0
	P (2-tailed)	0.090	0.241	0.046	<0.001
	N	5	5	5	5

*Correlation is significant at P <0.05 (2-tailed).

**Correlation is significant at P <0.01 (2-tailed).

Appendix 3.7: Mean growth parameters of *D. regia* in crude oil-contaminated soil at 2 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	7.72±6.11	0.08±0.04	2.00±1.14
25	0.00±0.00	0.00±0.00	0.00±0.00
50	0.00±0.00	0.00±0.00	0.00±0.00
75	0.00±0.00	0.00±0.00	0.00±0.00
100	0.00±0.00	0.00±0.00	0.00±0.00

*Weeks after planting.

Appendix 3.8: Mean growth parameters of *B. monandra* in crude oil-contaminated soil at 2 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	0.76±1.70	0.02±0.04	0.00±0.45
25	0.00±0.00	0.00±0.00	0.00±0.00
50	0.00±0.00	0.00±0.00	0.00±0.00
75	0.00±0.00	0.00±0.00	0.00±0.00
100	0.00±0.00	0.00±0.00	0.00±0.00

*Weeks after planting.

Appendix3.9: Mean growth parameters of *T. tetraptera* in crude oil-contaminated soil at 2 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	1.3±0.84	0.08±0.04	1.00±0.71
25	1.20±0.76	0.08±0.04	1.00±0.45
50	0.76±0.77	0.06±0.05	1.00±0.55
75	0.24±0.54	0.02±0.04	0.00±0.00
100	0.00±0.00	0.00±0.00	0.00±0.00

*Weeks after planting.

Appendix3.10: Mean growth parameters of *D. regia* in crude oil-contaminated soil at 4 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	22.30±6.58	0.16±0.05	2.00±0.84
25	2.90±3.04	0.06±0.05	0.00±0.00
50	1.46±2.02	0.04±0.05	0.00±0.55
75	0.40±0.89	0.0±0.00	0.00±0.00
100	0.00±0.00	0.00±0.00	0.00±0.00

*Week after planting.

Appendix 3.11: Mean growth parameters of *B. monandra* in crude oil-contaminated soil at 4 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	8.50±2.93	0.12±0.04	2.00±0.71
25	1.22±2.73	0.02±0.04	0.00±0.55
50	0.00±0.00	0.00±0.00	0.00±0.00
75	0.00±0.00	0.00±0.00	0.00±0.00
100	0.00±0.00	0.00±0.00	0.00±0.00

*Week after planting.

Appendix 3.12: Mean growth parameters of *T. tetraptera* in crude oil-contaminated soil at 4 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	2.46±0.48	0.10±0.00	1.00±0.55
25	2.30±0.55	0.10±0.00	2.00±0.55
50	1.86±0.57	0.10±0.00	1.00±0.45
75	0.80±1.13	0.04±0.05	1.00±0.89
100	0.00±0.67	0.00±0.05	0.00±0.55

*Week after planting.

Appendix 3.13: Mean growth parameters of *D. regia* in crude oil-contaminated soil at 6 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	39.10±7.39	0.34±0.11	4.00±1.14
25	15.82±8.50	0.16±0.05	3.00±1.17
50	12.78±4.51	0.14±0.05	2.00±0.84
75	5.52±4.93	0.08±0.00	1.00±0.50
100	4.02±0.74	0.01±0.00	1.00±0.00

*Week after planting.

Appendix 3.14: Mean growth parameters of *B. monandra* in crude oil-contaminated soil at 6 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	21.70±3.74	0.22±0.04	5.00±1.14
25	10.00±4.31	0.12±0.04	2.00±0.71
50	1.36±1.98	0.04±0.00	0.00±0.00
75	0.66±1.01	0.04±0.05	0.00±0.45
100	0.31±0.93	0.02±0.05	0.00±0.45

*Week after planting.

Appendix 3.15: Mean growth parameters of *T. tetraptera* in crude oil-contaminated soil at 6 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	5.88±0.34	0.10±0.00	5.00±0.55
25	4.84±0.55	0.10±0.00	4.00±0.00
50	4.40±0.75	0.10±0.00	3.00±0.84
75	3.08±1.19	0.12±0.00	2.00±1.05
100	2.98±1.08	0.10±0.00	2.00±0.84

*Week after planting.

Appendix 3.16: Mean growth parameters of *D. regia* in crude oil-contaminated soil at 8 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	55.00±7.89	0.46±0.11	8.00±1.34
25	28.94±8.07	0.24±0.05	5.00±1.30
50	26.38±4.57	0.24±0.05	4.00±1.00
75	20.32±6.47	0.20±0.07	3.00±0.71
100	7.69±1.15	0.50±0.00	1.00±0.00

*Week after planting.

Appendix 3.17: Mean growth parameters of *B. monandra* in crude oil-contaminated soil at 8 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	34.54±4.34	0.36±0.05	7.00±1.58
25	23.80±5.75	0.26±0.05	4.00±1.00
50	12.72±2.73	0.16±0.05	2.00±0.55
75	10.44±2.31	0.12±0.04	2.00±0.55
100	8.82±4.35	0.10±0.00	1.00±0.45

*Week after planting.

Appendix 3.18: Mean growth parameters of *T. tetraptera* in crude oil-contaminated soil at 8 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	10.00±0.59	0.20±0.00	6.00±0.71
25	7.48±1.37	0.12±0.04	4.00±0.55
50	7.36±1.44	0.12±0.04	4.00±0.89
75	6.18±1.35	0.10±0.00	3.00±0.89
100	5.28±0.68	0.10±0.00	2.00±0.89

*Week after planting.

Appendix 3.19: Mean growth parameters of *D. regia* in crude oil-contaminated soil at 10 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	65.70±13.27	0.38±0.13	13.00±1.92
25	41.08±11.86	0.38±0.13	9.00±3.67
50	41.04±4.62	0.30±0.10	7.00±1.10
75	32.86±5.76	0.28±0.08	5.00±1.34
100	25.36±1.88	0.20±0.00	4.00±0.45

*Week after planting.

Appendix 3.20: Mean growth parameters of *B. monandra* in crude oil-contaminated soil at 10 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	49.02±4.32	0.64±0.09	9.00±1.30
25	39.24±4.17	0.42±0.04	6.00±0.84
50	25.68±3.31	0.32±0.14	4.00±0.84
75	23.46±3.38	0.24±0.05	3.00±0.55
100	20.72±4.67	0.12 ±0.04	3.00±0.55

*Week after planting.

Appendix 3.21: Mean growth parameters of *T. tetraptera* in crude oil-contaminated soil at 10 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	13.04±0.63	0.24±0.05	8.00±0.89
25	10.56±2.03	0.20±0.07	5.00±1.58
50	10.40±0.29	0.18±0.04	5.00±0.45
75	9.16±1.33	0.12 ±0.04	3.00±1.00
100	8.20±0.23	0.10±0.00	3.00±0.55

*Week after planting.

Appendix 3.22: Mean growth parameters of *D. regia* in crude oil-contaminated soil at 12 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	84.12±8.31	0.78±0.08	17.00±1.67
25	58.12±6.77	0.60±0.00	13.00±1.34
50	51.76±4.90	0.46±0.05	9.00±1.30
75	43.90±3.93	0.36±0.05	6.00±0.84
100	35.04±1.77	0.26±0.05	4.00±0.55

*Week after planting.

Appendix 3.23: Mean growth parameters of *B. monandra* in crude oil-contaminated soil at 12 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	60.54±5.50	0.70±0.14	10.00±1.79
25	51.60±4.67	0.48±0.08	7.00±0.84
50	37.98±2.76	0.24±0.13	4.00±0.89
75	33.84±3.54	0.30±0.00	3.00±0.45
100	30.48±3.83	0.16±0.05	4.00±0.89

*Week after planting.

Appendix 3.24: Mean growth parameters of *T. tetraptera* in crude oil-contaminated soil at 12 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	15.64±0.83	0.40±0.00	8.00±0.45
25	13.00±2.08	0.20±0.05	7.00±1.52
50	13.26±0.62	0.28±0.04	6.00±0.71
75	11.62±0.97	0.20±0.00	4.00±0.84
100	10.66±0.65	0.16±0.05	3.00±0.00

*Week after planting.

Appendix 3.25: Mean growth parameters of *D. regia* in crude oil-contaminated soil at 14 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	98.40±13.16	0.88±0.11	18.00±2.45
25	70.50±6.40	0.74±0.05	14.00±1.64
50	64.62±5.04	0.58±0.04	11.00±2.41
75	54.64±4.84	0.44±0.09	8.00±1.14
100	45.14±1.24	0.30±0.00	5.00±0.45

*Week after planting.

Appendix 3.26: Mean growth parameters of *B. monandra* in crude oil-contaminated soil at 14 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	75.90±3.67	0.78±0.04	12.00±1.30
25	63.04±4.64	0.60±0.07	8.00±0.89
50	49.66±4.02	0.42±0.05	5.00±0.45
75	43.54±4.51	0.38±0.08	4.00±1.30
100	41.18±4.33	0.30±0.10	4.00±0.89

*Week after planting.

Appendix 3.27: Mean growth parameters of *T. tetraptera* in crude oil-contaminated soil at 14 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	18.36±1.34	0.38±0.04	8.00±1.10
25	15.38±1.97	0.28±0.04	7.00±1.10
50	15.06±0.66	0.30±0.00	6.00±1.14
75	13.64±1.06	0.20±0.00	4.00±0.84
100	11.94±0.40	0.14±0.05	3.00±0.00

*Week after planting.

Appendix 3.28: Mean growth parameters of *D. regia* in crude oil-contaminated soil at 16 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	114.20±10.34	1.10±0.07	23.00±1.73
25	79.18±10.68	0.72±0.13	15.00±3.58
50	78.90±4.84	0.70±0.04	13.00±1.87
75	65.68±5.54	0.56±0.09	10.00±1.52
100	55.36±0.78	0.44±0.04	6.00±0.71

*Week after planting.

Appendix 3.29: Mean growth parameters of *B. monandra* in crude oil-contaminated soil at 16 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	91.26±2.66	0.88±0.04	14.00±1.52
25	74.58±4.44	0.70±0.07	9.00±0.89
50	61.10±2.92	0.54±0.05	6.00±0.84
75	53.88±3.90	0.44±0.05	6.00±0.89
100	51.32±4.78	0.42±0.11	4.00±0.89

*Week after planting.

Appendix 3.30: Mean growth parameters of *T. tetraptera* in crude oil-contaminated soil at 16 WAP*

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	21.16±0.97	0.40±0.04	9.00±0.55
25	17.86±1.24	0.34±0.05	7.00±1.00
50	16.80±0.62	0.30±0.04	7.00±0.84
75	15.34±1.23	0.20±0.04	5.00±1.14
100	13.90±0.58	0.16±0.05	3.00±0.55

*Week after planting.

Appendix 3.31: Correlation analysis of mean seedling heights of leguminous tree species (LTS) at 16 WAP

LTS		Treatments	Height of *D. regia*	Height of *B. monandra*	Height of *T. tetraptera*
Treatments	Pearson Correlation	1.0	-0.885**	-0.936**	-0.921**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Height of *D. regia*	Pearson Correlation	-0.885**	1.0	0.868**	0.899**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Height of *B. monandra*	Pearson Correlation	-0.936**	0.868**	1.0	0.927**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Height of *T. tetraptera*	Pearson Correlation	-0.921**	0.899**	0.927**	1.0
	P (2-tailed)	<0.001	0.001	<0.001	<0.001
	N	25	25	25	25

**Correlation is significant at P <0.01 (2-tailed).

Appendix 3.32: Correlation analysis of mean seedling girths of leguminous tree species (LTS) at 16 WAP

LTS		Treatments	Girth of *D. regia*	Girth of *B. monandra*	Girth of *T. tetraptera*
Treatments	Pearson Correlation	1.0	-0.898**	-0.905**	-0.890**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Girth of *D. regia*	Pearson Correlation	-0.898**	1.0	0.799**	0.841**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Girth of *B. monandra*	Pearson Correlation	-0.905**	0.799**	1.0	0.852**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Girth of *T. tetraptera*	Pearson Correlation	-0.890**	0.841**	0.852**	1.0
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25

**Correlation is significant at P <0.01 (2-tailed).

Appendix 3.33: Correlation analysis of mean leaf production of leguminous tree species (LTS) at 16 WAP

LTS		Treatments	Number of Leaves of *D. regia*	Number of Leaves of *B. monandra*	Number of Leaves of *T. tetraptera*
Treatments	Pearson Correlation	1.0	-0.930**	-0.900**	-0.920**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Number of Leaves *D. regia*	Pearson Correlation	-0.930**	1.0	0.874**	0.849**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Number of Leaves *B. monandra*	Pearson Correlation	-0.900**	0.874**	1.0	0.830**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Number of Leaves *T. tetraptera*	Pearson Correlation	-0.920**	0.849**	0.830**	1.0
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25

**Correlation is significant at the 0.01 level (2-tailed).

Appendix 3.34: ANOVA of leguminous tree species shoot and root biomass (total plant biomass) at 16 WAP

		Sum of Squares	df	Mean Square	F	P
Shoots of *D. regia*	Between Groups	9943.377	4	2485.844	48.110	<0.001
	Within Groups	516.700	10	51.670		
	Total	10460.077	14			
Roots of *D. regia*	Between Groups	865.220	4	216.305	72.977	<0.001
	Within Groups	29.640	10	2.964		
	Total	894.860	14			
Shoots of *B. monandra*	Between Groups	5259.637	4	1314.909	37.409	<0.001
	Within Groups	351.500	10	35.150		
	Total	5611.137	14			
Roots of *B. monandra*	Between Groups	293.467	4	73.367	66.495	<0.001
	Within Groups	11.033	10	1.103		
	Total	304.500	14			
Shoots of *T. tetraptera*	Between Groups	112.903	4	28.226	34.062	<0.001
	Within Groups	8.287	10	0.829		
	Total	121.189	14			
Roots of *T. tetraptera*	Between Groups	5.549	4	1.387	3.125	<0.001
	Within Groups	4.440	10	0.444		
	Total	9.989	14			

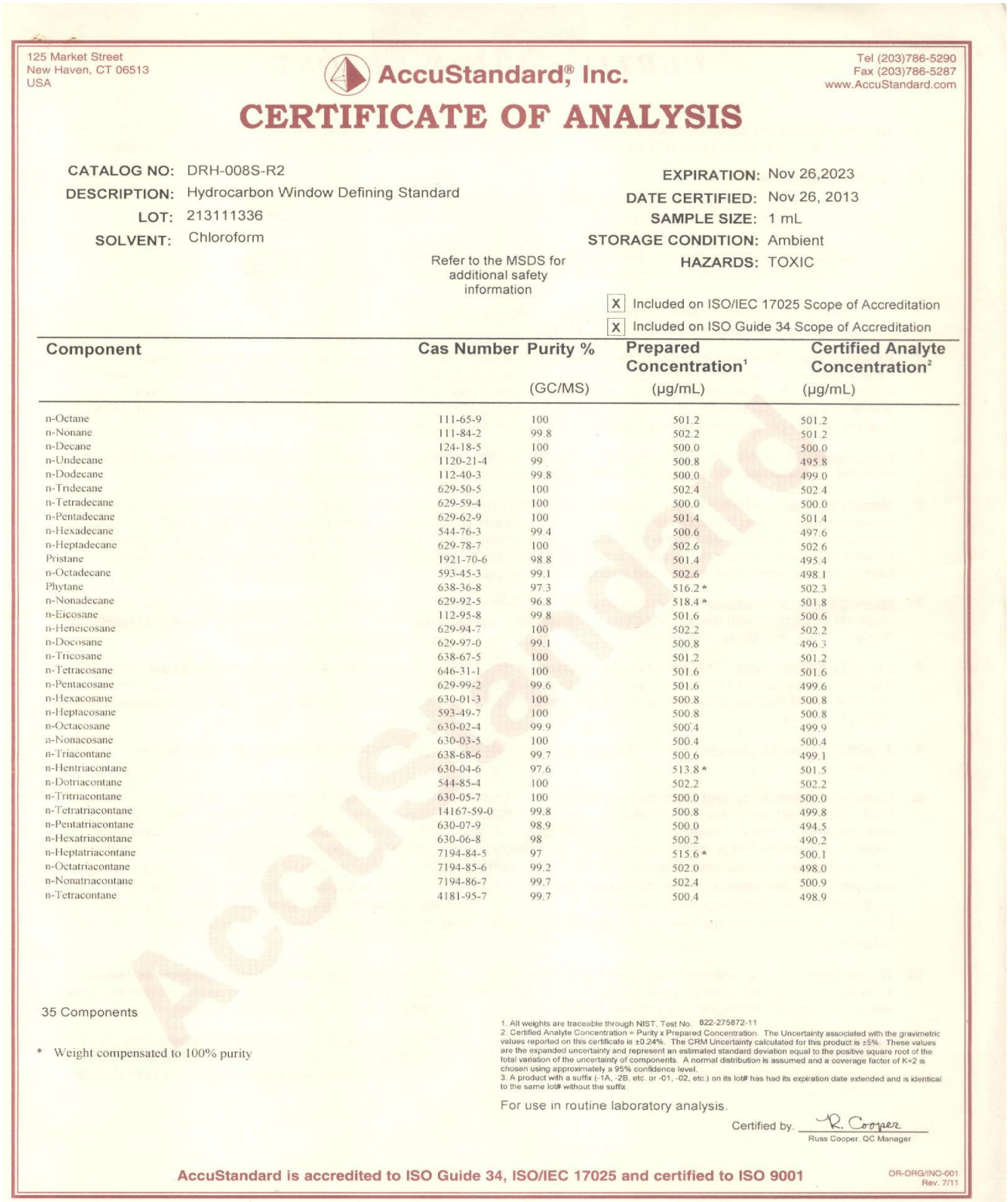
Appendix 3.35: ANOVA of microbial counts in crude oil-contaminated soil planted with leguminous tree species (LTS) at 16 WAP

Soil planted with LTS		Sum of Squares	df	Mean Square	F	P
Soil with *D. regia* Number of HBC†	Between Groups	5718.933	4	1429.733	14.184	<0.001
	Within Groups	1008.000	10	100.800		
	Total	6726.933	14			
Soil with *D. regia* Number of HFC††	Between Groups	972.000	4	243.000	7.500	0.005
	Within Groups	324.000	10	32.400		
	Total	1296.000	14			
Soil with *B. monandra* Number of HBC†	Between Groups	4399.067	4	1099.767	10.059	0.002
	Within Groups	1093.333	10	109.333		
	Total	5492.400	14			
Soil with *B. monandra* Number of HFC††	Between Groups	557.733	4	139.433	2.901	0.078
	Within Groups	480.667	10	48.067		
	Total	1038.400	14			
Soil with *T. tetraptera* Number of HBC†	Between Groups	1303.067	4	325.767	2.755	0.088
	Within Groups	1182.667	10	118.267		
	Total	2485.733	14			
Soil with *T. tetraptera* Number of HFC††	Between Groups	567.600	4	141.900	2.994	0.073
	Within Groups	474.000	10	47.400		
	Total	1041.600	14			

†Heterotrophic Bacterial Counts

††Heterotrophic Fungi Counts.

Appendix 3.36: Aliphatic hydrocarbon compounds detectable by the TPH standard in the crude oil-contaminated soil



125 Market Street
New Haven, CT 06513
USA

AccuStandard® Inc.

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CERTIFICATE OF ANALYSIS

CATALOG NO: DRH-008S-R2
DESCRIPTION: Hydrocarbon Window Defining Standard
LOT: 213111336
SOLVENT: Chloroform

EXPIRATION: Nov 26,2023
DATE CERTIFIED: Nov 26, 2013
SAMPLE SIZE: 1 mL
STORAGE CONDITION: Ambient
HAZARDS: TOXIC

Refer to the MSDS for additional safety information

[X] Included on ISO/IEC 17025 Scope of Accreditation
[X] Included on ISO Guide 34 Scope of Accreditation

Component	Cas Number	Purity % (GC/MS)	Prepared Concentration[1] (µg/mL)	Certified Analyte Concentration[2] (µg/mL)
n-Octane	111-65-9	100	501.2	501.2
n-Nonane	111-84-2	99.8	502.2	501.2
n-Decane	124-18-5	100	500.0	500.0
n-Undecane	1120-21-4	99	500.8	495.8
n-Dodecane	112-40-3	99.8	500.0	499.0
n-Tridecane	629-50-5	100	502.4	502.4
n-Tetradecane	629-59-4	100	500.0	500.0
n-Pentadecane	629-62-9	100	501.4	501.4
n-Hexadecane	544-76-3	99.4	500.6	497.6
n-Heptadecane	629-78-7	100	502.6	502.6
Pristane	1921-70-6	98.8	501.4	495.4
n-Octadecane	593-45-3	99.1	502.6	498.1
Phytane	638-36-8	97.3	516.2 *	502.3
n-Nonadecane	629-92-5	96.8	518.4 *	501.8
n-Eicosane	112-95-8	99.8	501.6	500.6
n-Heneicosane	629-94-7	100	502.2	502.2
n-Docosane	629-97-0	99.1	500.8	496.3
n-Tricosane	638-67-5	100	501.2	501.2
n-Tetracosane	646-31-1	100	501.6	501.6
n-Pentacosane	629-99-2	99.6	501.6	499.6
n-Hexacosane	630-01-3	100	500.8	500.8
n-Heptacosane	593-49-7	100	500.8	500.8
n-Octacosane	630-02-4	99.9	500.4	499.9
n-Nonacosane	630-03-5	100	500.4	500.4
n-Triacontane	638-68-6	99.7	500.6	499.1
n-Hentriacontane	630-04-6	97.6	513.8 *	501.5
n-Dotriacontane	544-85-4	100	502.2	502.2
n-Tritriacontane	630-05-7	100	500.0	500.0
n-Tetratriacontane	14167-59-0	99.8	500.8	499.8
n-Pentatriacontane	630-07-9	98.9	500.0	494.5
n-Hexatriacontane	630-06-8	98	500.2	490.2
n-Heptatriacontane	7194-84-5	97	515.6 *	500.1
n-Octatriacontane	7194-85-6	99.2	502.0	498.0
n-Nonatriacontane	7194-86-7	99.7	502.4	500.9
n-Tetracontane	4181-95-7	99.7	500.4	498.9

35 Components

* Weight compensated to 100% purity

1. All weights are traceable through NIST, Test No. 822-275872-11
2. Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty associated with the gravimetric values reported on this certificate is ±0.24%. The CRM Uncertainty calculated for this product is ±5%. These values are the expanded uncertainty and represent an estimated standard deviation equal to the positive square root of the total variation of the uncertainty of components. A normal distribution is assumed and a coverage factor of K=2 is chosen using approximately a 95% confidence level.
3. A product with a suffix (-1A, -2B, etc. or -01, -02, etc.) on its lot# has had its expiration date extended and is identical to the same lot# without the suffix.

For use in routine laboratory analysis.

Certified by: R. Cooper
Russ Cooper, QC Manager

AccuStandard is accredited to ISO Guide 34, ISO/IEC 17025 and certified to ISO 9001

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Appendix3.37: Polyaromatic hydrocarbon compounds detectable by the TPH standard in the crude oil-contaminated soil

125 Market Street
New Haven, CT 06513
USA

AccuStandard® Inc.

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CERTIFICATE OF ANALYSIS

CATALOG NO: Z-014G-R
DESCRIPTION: PAH Mix
LOT: 214011262
SOLVENT: Dichloromethane
Benzene

Refer to the MSDS for additional safety information

EXPIRATION: Jan 23,2024
DATE CERTIFIED: Jan 23, 2014
SAMPLE SIZE: 1 mL
STORAGE CONDITION: Ambient
HAZARDS: FLAMMABLE/TOXIC

[X] Included on ISO/IEC 17025 Scope of Accreditation
[X] Included on ISO Guide 34 Scope of Accreditation

Component	Cas Number	Purity % (GC/MS)	Prepared Concentration[1] (µg/mL)	Certified Analyte Concentration[2] (µg/mL)
Acenaphthene	83-32-9	100	2002	2002
Acenaphthylene	208-96-8	99.2	2000	1984
Anthracene	120-12-7	99.6	2007	1999
Benz(a)anthracene	56-55-3	100	2003	2003
Benzo(a)pyrene	50-32-8	99.8	2011	2007
Benzo(b)fluoranthene	205-99-2	99.7	2010	2004
Benzo(g,h,i)perylene	191-24-2	98.9	2004	1982
Benzo(k)fluoranthene	207-08-9	99.1	2005	1987
Chrysene	218-01-9	99.8	2009	2005
Dibenz(a,h)anthracene	53-70-3	99.0	2001	1981
Fluoranthene	206-44-0	97.2	2058 *	2000
Fluorene	86-73-7	98.1	2004	1966
Indeno(1,2,3-cd)pyrene	193-39-5	99.2	2013	1997
Naphthalene	91-20-3	99.5	2005	1995
Phenanthrene	85-01-8	99.5	2014	2004
Pyrene	129-00-0	99.1	2001	1983
Carbazole	86-74-8	99.7	2000	1994

17 Components

* Weight compensated to 100% purity

1. All weights are traceable through NIST, Test No. 822-275872-11
2. Certified Analyte Concentration = Purity x Prepared Concentration. The Uncertainty associated with the gravimetric values reported on this certificate is ±0.24%. The CRM Uncertainty calculated for this product is ±5%. These values are the expanded uncertainty and represent an estimated standard deviation equal to the positive square root of the total variation of the uncertainty of components. A normal distribution is assumed and a coverage factor of K=2 is chosen using approximately a 95% confidence level.
3. A product with a suffix (-1A, -2B, etc. or -01, -02, etc.) on its lot# has had its expiration date extended and is identical to the same lot# without the suffix.

For use in routine laboratory analysis.

Certified by: R. Cooper
Russ Cooper, QC Manager

AccuStandard is accredited to ISO Guide 34, ISO/IEC 17025 and certified to ISO 9001

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Appendix 3.38: ANOVA of mean TPH in crude oil-contaminated soil remediated with leguminous tree species (LTS) at 16 WAP*

Treatment		Sum of Squares	df	Mean Square	F	P
TPH Non-planted soil	Between Groups	1268.419	3	422.806	1.437	0.302
	Within Groups	2353.907	8	294.238		
	Total	3622.326	11			
TPH Soil planted with *D. regia*	Between Groups	135.868	3	45.289	16.341	<0.001
	Within Groups	22.172	8	2.772		
	Total	158.040	11			
TPH Soil planted with *B. monandra*	Between Groups	4564.719	3	1521.573	7.936	0.009
	Within Groups	1533.799	8	191.725		
	Total	6098.518	11			
TPH Soil planted with *T. tetraptera*	Between Groups	87.477	3	29.159	3.671	0.063
	Within Groups	63.553	8	7.944		
	Total	151.030	11			

Appendix 4.1a: ANOVA between contamination and mean germination of Leguminous Tree Species (LTS)

Treatment		Sum of Squares	df	Mean Square	F	P
Germination of *P. pterocarpum*	Between Groups	32.160	4	8.040	14.889	<0.001
	Within Groups	10.800	20	0.540		
	Total	42.960	24			
Germination of *A. odoratissima*	Between Groups	84.160	4	21.040	18.456	<0.001
	Within Groups	22.800	20	1.140		
	Total	106.960	24			
Germination of *A. adianthifolia*	Between Groups	50.640	4	12.660	19.781	<0.001
	Within Groups	12.800	20	0.640		
	Total	63.440	24			

Appendix 4.1b: ANOVA between contamination and Co-efficient of Velocity (COV) of germination of LTS seeds

Treatment		Sum of Squares	df	Mean Square	F	P
COV of *P. pterocarpum*	Between Groups	7.186	4	1.796	11.795	<0.001
	Within Groups	3.046	20	0.152		
	Total	10.232	24			
COV of *A. odoratissima*	Between Groups	19.141	4	4.785	20.930	<0.001
	Within Groups	4.573	20	0.229		
	Total	23.713	24			
COV of *A. adianthifolia*	Between Groups	41.053	4	10.263	3.731	0.020
	Within Groups	55.019	20	2.751		
	Total	96.072	24			

Appendix 4.2: Correlations between contamination and germination of LTS

Treatment		Treatments	Germination of *P. pterocarpum*	Germination of *A. odoratissima*	Germination of *A. adianthifolia*
Treatments	Pearson Correlation	1.0	-0.841**	-0.875**	-0.888**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Germination of *P. pteroparpum*	Pearson Correlation	-0.841**	1.0	0.693**	0.856**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Germination of *A. odoratissima*	Pearson Correlation	-0.875**	0.693**	1.0	0.809**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	N	25	25	25	25
Germination of *A. adianthifolia*	Pearson Correlation	-0.888**	0.856**	0.809**	1.0
	P (2-tailed)	<0.001	<0.001	0.001	<0.001
	N	25	25	25	25

**Correlation is significant at P <0.01 (2-tailed).

Appendix 4.3: Mean growth parameters of *P. pterocarpum* in crude oil-contaminated soil at 2WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	3.22±2.07	0.08±0.04	1.00±0.45
25	1.68±1.66	0.06±0.05	1.00±0.55
50	0.70±1.57	0.02±0.04	0.00±0.45
75	7.72.±6.11	0.08±0.04	2.00±1.14
100	0.48±1.07	0.02±0.07	0.00±0.45

[#]Weeks after planting.

Appendix 4.4: Mean growth parameters of *A. odoratissima* in crude oil-contaminated soil at 2WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	5.12±1.03	0.01±0.00	2.00±0.55
25	3.18±1.87	0.08±0.04	1.00±0.71
50	1.76±1.65	0.06±0.05	1.00±0.55
75	1.10±1.57	0.04±0.05	0.00±0.55
100	0.00±0.00	0.00±0.00	0.00±0.00

[#]Weeks after planting.

Appendix 4.5: Mean growth parameters of *A. adianthifolia* in crude oil-contaminated soil at 2WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	1.54±1.52	0.06±0.05	0.20±0.45
25	0.48±1.07	0.02±0.04	0.20±0.45
50	0.00±0.00	0.00±0.00	0.00±0.00
75	0.00±0.00	0.00±0.00	0.00±0.00
100	0.00±0.00	0.00±0.00	0.00±0.00

[#]Weeks after planting.

Appendix 4.6: Mean growth parameters of *P. pterocarpum* in crude oil-contaminated soil at 4WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	7.84±3.43	0.10±0.03	3.00±1.00
25	9.36±1.77	0.12±0.04	2.00±0.45
50	9.16±1.80	0.12±0.04	2.00±0.55
75	6.38±2.31	0.10±0.00	1.00±0.45
100	5.44±1.18	0.10±0.00	1.00±0.55

[#]Weeks after planting.

Appendix 4.7: Mean growth parameters of *A. odoratissima* on contaminated soil at 4 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	7.66±0.57	0.01±0.00	4.00±1.53
25	5.80±0.82	0.01±0.00	2.00±0.89
50	5.00±0.99	0.01±0.00	1.00±0.55
75	4.66±0.71	0.01±0.00	1.00±0.55
100	3.56±0.58	0.01±0.00	1.00±0.00

[*]Weeks after planting.

Appendix 4.8: Mean growth parameters of *A. adianthifolia* on contaminated soil at 4WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	10.06±1.08	0.01±0.00	3.00±0.71
25	7.92±1.19	0.01±0.00	2.00±0.71
50	5.74±0.78	0.01±0.00	1.00±0.00
75	5.78±1.14	0.01±0.00	1.00±0.00
100	4.94±0.54	0.01±0.00	1.00±0.00

[#]Weeks after planting.

Appendix 4.9: Mean growth parameters of *P. pterocarpum* in crude oil-contaminated soil at 6 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	18.70±2.67	0.24±0.05	4.00±1.30
25	15.64±2.17	0.20±0.00	3.00±1.00
50	14.54±1.88	0.20±0.00	3.00±0.55
75	12.64±1.75	0.12±0.04	2.00±0.45
100	13.20±0.90	0.12±0.04	2.00±0.00

[#]Weeks after planting.

Appendix 4.10: Mean growth parameters of A. odoratissima on contaminated soil at 6 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	12.04±1.51	0.20±0.00	4.00±0.00
25	9.62±0.72	0.16±0.05	3.00±0.71
50	8.00±1.31	0.10±0.00	2.00±0.45
75	7.26±1.44	0.10±0.00	2.00±0.00
100	6.80±0.64	0.10±0.00	2.00±0.00

[#]Weeks after planting.

Appendix 4.11: Mean growth parameters of *A. adianthifolia* on contaminated soil at 6 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	19.14±2.10	0.20±0.00	5.00±1.64
25	14.88±1.30	0.14±0.05	3.00±0.84
50	14.30±1.42	0.10±0.00	2.00±0.89
75	12.92±1.75	0.10±0.00	2.00±0.00
100	13.02±1.94	0.10±0.00	2.00±0.00

[#]Weeks after planting.

Appendix 4.12: Mean growth parameters of *P. pterocarpum* in crude oil-contaminated soil at 8 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	28.12±3.56	0.26±0.05	4.00±1.14
25	24.84±2.77	0.20±0.00	4.00±0.89
50	23.58±1.58	0.20±0.00	3.00±0.71
75	20.58±3.22	0.20±0.00	3.00±0.55
100	23.00±0.86	0.20±0.00	3.00±0.45

[*]Weeks after planting.

Appendix 4.13: Mean growth parameters of *A. odoratissima* on contaminated soil at 8 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	18.92±1.52	0.20±0.00	6.00±1.10
25	16.02±1.17	0.20±0.00	4.00±0.55
50	13.62±0.73	0.18±0.04	4.00±0.55
75	12.12±0.79	0.12±0.04	2.00±0.00
100	10.34±0.61	0.10±0.00	2.00±0.00

[#]Weeks after planting.

Appendix 4.14: Mean growth parameters of *A. adianthifolia* on contaminated soil at 8 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	28.44±1.40	0.22±0.04	6.00±1.52
25	25.56±1.78	0.20±0.00	3.00±0.71
50	23.18±2.18	0.12±0.04	2.00±0.45
75	22.68±1.07	0.10±0.00	2.00±0.00
100	20.68±2.20	0.12±0.04	2.00±0.00

[#]Weeks after planting.

Appendix 4.15: Mean growth parameters of *P. pterocarpum* in crude oil-contaminated soil at 10 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	43.50±3.68	0.42±0.04	7.00±1.87
25	36.14±2.44	0.32±0.04	6.00±0.45
50	34.24±1.47	0.28±0.04	5.00±0.45
75	30.70±0.51	0.20±0.00	4.00±0.55
100	30.40±1.10	0.20±0.00	4.00±0.45

[#]Weeks after planting.

Appendix 4.16: Mean growth parameters of *A. odoratissima* in crude oil-contaminated soil at 10 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	35.64±1.65	0.32±0.04	6.00±1.00
25	30.00±2.50	0.24±0.05	4.00±0.45
50	23.88±0.41	0.20±0.00	4.00±0.55
75	23.18±1.13	0.20±0.00	2.00±0.55
100	21.30±0.57	0.20±0.00	2.00±0.45

[#]Weeks after planting.

Appendix 4.17: Mean growth parameters of *A. adianthifolia* in crude oil-contaminated soil at 10 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	42.56±2.96	0.36±0.05	7.00±0.71
25	33.98±2.47	0.28±0.04	4.00±0.04
50	33.22±2.55	0.24±0.05	3.00±0.45
75	29.78±1.98	0.18±0.04	2.00±0.00
100	27.20±3.02	0.16±0.00	2.00±0.00

[#]Weeks after planting.

Appendix 4.18: Mean growth parameters of *P. pterocarpum* in crude oil-contaminated soil at 12 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	57.80±3.79	0.58±0.11	13.00±2.19
25	51.56±4.13	0.46±0.05	9.00±1.30
50	46.42±2.64	0.38±0.04	7.00±0.89
75	49.34±1.22	0.32±0.04	6.00±0.45
100	43.24±2.46	0.26±0.45	5.00±0.45

[#]Weeks after planting.

Appendix 4.19: Mean growth parameters of *A. odoratissima* in crude oil-contaminated soil at 12 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	52.56±1.39	0.38±0.04	8.00±0.89
25	44.16±2.57	0.38±0.04	6.00±0.55
50	36.98±0.71	0.30±0.00	5.00±0.45
75	35.42±1.69	0.26±0.05	2.00±0.55
100	32.62±1.61	0.20±0.00	4.00±0.55

[#]Weeks after planting.

Appendix 4.20: Mean growth parameters of *A. adianthifolia* in crude oil-contaminated soil at 12 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	58.46±4.86	0.48±0.04	8.00±0.84
25	46.20±2.34	0.34±0.05	4.00±0.55
50	44.00±2.08	0.32±0.04	3.00±0.45
75	39.64±2.28	0.22±0.04	3.00±0.55
100	36.26±1.22	0.20±0.00	3.00±0.45

[#]Weeks after planting.

Appendix 4.21: Mean growth parameters of *P. pterocarpum* in crude oil-contaminated soil at 14 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	81.26±3.71	0.76±0.05	20.00±2.49
25	70.38±3.56	0.58±0.04	14.00±0.84
50	64.60±3.67	0.56±0.05	7.00±0.89
75	63.04±2.35	0.52±0.04	11.00±1.30
100	56.86±7.28	0.44±0.09	10.00±1.34

[#]Weeks after planting.

Appendix 4.22: Mean growth parameters of *A. odoratissima* in crude oil-contaminated soil at 14 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	64.68±3.73	0.66±0.13	13.00±1.67
25	59.30±1.50	0.53±0.04	10.00±0.84
50	48.88±1.43	0.40±0.00	8.00±1.48
75	46.90±0.80	0.38±0.04	2.00±0.55
100	43.48±2.77	0.32±0.04	4.00±0.55

[#]Weeks after planting.

Appendix 4.23: Mean growth parameters of *A. adianthifolia* in crude oil-contaminated soil at 14 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	70.80±6.05	0.56±0.09	11.00±1.58
25	57.96±2.41	0.36±0.05	6.00±1.34
50	53.58±2.27	0.34±0.05	3.00±0.45
75	51.44±1.67	0.28±0.04	5.00±1.52
100	43.78±1.14	0.20±0.00	3.00±0.55

[#]Weeks after planting.

Appendix 4.24: Mean growth parameters of *P. pterocarpum* in crude oil-contaminated soil at 16 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	89.58±3.61	0.82±0.04	23.00±2.68
25	76.68±3.94	0.68±0.04	16.00±1.22
50	71.38±3.68	0.62±0.04	14.00±1.10
75	73.28±7.59	0.58±0.04	13.00±1.52
100	63.64±7.61	0.50±0.07	11.00±1.64

[#]Weeks after planting.

Appendix 4.25: Mean growth parameters of *A. odoratissima* in crude oil-contaminated soil at 16 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	78.76±5.44	0.66±0.05	15.00±2.19
25	72.26±2.93	0.54±0.05	12.00±1.14
50	63.62±3.37	0.46±0.05	10.00±0.55
75	57.28±1.03	0.40±0.00	8.00±0.84
100	54.94±3.33	0.32±0.04	4.00±0.45

[#]Weeks after planting

Appendix 4.26: Mean growth parameters of *A. adianthifolia* in crude oil-contaminated soil at 16 WAP[#]

Treatment (ml)	Growth parameters		
	Height (cm)	Girth (cm)	Number of leaves
0	81.04±5.88	0.64±0.09	12.00±1.52
25	68.10±2.42	0.50±0.07	8.00±0.55
50	63.30±2.94	0.36±0.05	5.00±0.84
75	60.92±2.90	0.34±0.05	5.00±1.03
100	53.76±1.09	0.24±0.05	3.00±0.55

[#]Weeks after planting.

Appendix 4.27: ANOVA of selected growth parameters of LTS at 16 WAP[#]

		Sum of Squares	df	Mean Square	F	P
Height of P. *pterocarpum*	Between Groups	1802.362	4	450.591	14.294	<0.001
	Within Groups	630.464	20	31.523		
	Total	2432.826	24			
Girth of P. *pterocarpum*	Between Groups	0.288	4	0.072	27.692	<0.001
	Within Groups	0.052	20	0.003		
	Total	0.340	24			
Number of leaves of P. *pterocarpum*	Between Groups	410.160	4	102.540	34.409	<0.001
	Within Groups	59.600	20	2.980		
	Total	469.760	24			
Height of *A. odoratissima*	Between Groups	2020.298	4	505.075	40.938	<0.001
	Within Groups	246.752	20	12.338		
	Total	2267.050	24			
Girth of *A. odoratissima*	Between Groups	0.342	4	0.085	38.818	<0.001
	Within Groups	0.044	20	0.002		
	Total	0.386	24			
Number of leaves of *A. odoratissima*	Between Groups	233.040	4	58.260	39.904	<0.001
	Within Groups	29.200	20	1.460		
	Total	262.240	24			
Height of *A. adianthifolia*	Between Groups	2059.334	4	514.833	43.877	<0.001
	Within Groups	234.672	20	11.734		
	Total	2294.006	24			
Girth of *A. adianthifolia*	Between Groups	0.486	4	0.121	27.591	<0.001
	Within Groups	0.088	20	0.004		
	Total	0.574	24			
Number of leaves of *A. adianthifolia*	Between Groups	256.800	4	64.200	60.566	<0.001
	Within Groups	21.200	20	1.060		
	Total	278.000	24			

[#]Weeks After Planting (WAP).

Appendix 4.28: Correlations among LTS mean seedling heights at 16 WAP[#]

LTS		Treatments	Height of *P. pterocarpum*	Height of *A. odoratissima*	Height of *A. adianthifolia*
Treatments	Pearson Correlation	1.0	-0.792**	-0.930**	-0.911**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	50.000	-276.400	-313.100	-308.700
	Covariance	2.083	-11.517	-13.046	-12.862
	N	25	25	25	25
Height of *P. pterocarpum*	Pearson Correlation	-0.792**	1.0	0.766**	0.816**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-276.400	2432.826	1799.228	1928.173
	Covariance	-11.517	101.368	74.968	80.341
	N	25	25	25	25
Height of *A. odoratissima*	Pearson Correlation	-0.930**	0.766**	1.0	.902**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-313.100	1799.228	2267.050	2057.117
	Covariance	-13.046	74.968	94.460	85.713
	N	25	25	25	25
Height of *A. adianthifolia*	Pearson Correlation	-0.911**	0.816**	0.902**	1.0
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-308.700	1928.173	2057.117	2294.006
	Covariance	-12.862	80.341	85.713	95.584
	N	25	25	25	25

**Correlation is significant at P <0.01 (2-tailed).
[#]Weeks After Planting (WAP).

Appendix 4.29: Correlations among LTS mean seedling girths at 16 WAP[#]

		Treatments	Girth of *P. pteroparpum*	Girth of *A. odoratissima*	Girth of *A. adianthifolia*
Treatments	Pearson Correlation	1.0	-0.897**	-0.934**	-0.896**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	50.000	-3.700	-4.100	-4.800
	Covariance	2.083	-0.154	-0.171	-0.200
	N	25	25	25	25
Girth of *P. pteroparpum*	Pearson Correlation	-0.897**	1.0	0.895**	0.892**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-3.700	0.340	0.324	0.394
	Covariance	-0.154	0.014	0.013	0.016
	N	25	25	25	25
Girth of *A. odoratissima*	Pearson Correlation	-0.934**	0.895**	1.0	0.913**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-4.100	0.324	0.386	0.430
	Covariance	-0.171	0.013	0.016	0.018
	N	25	25	25	25
Girth of *A. adianthifolia*	Pearson Correlation	-0.896**	0.892**	0.913**	1.0
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-4.800	0.394	0.430	0.574
	Covariance	-0.200	0.016	0.018	0.024
	N	25	25	25	25

**Correlation is significant at P <0.01 (2-tailed).
[#]Weeks After Planting (WAP).

Appendix 4.30: Correlations among LTS leaf production at 16 WAP[#]

		Treatments	Number of Leaves *P. pteroparpum*	Number of Leaves *A. odaratissima*	Number of Leaves *A. adianthifolia*
Treatments	Pearson Correlation	1.0	-0.868**	-0.917**	-0.882**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	50.000	-133.000	-105.000	-104.000
	Covariance	2.083	-5.542	-4.375	-4.333
	N	25	25	25	25
Number of Leaves *P. pteroparpum*	Pearson Correlation	-0.868**	1.0	0.828**	0.904**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-133.000	469.760	290.680	326.600
	Covariance	-5.542	19.573	12.112	13.608
	N	25	25	25	25
Number of Leaves *A. odaratissima*	Pearson Correlation	-0.917**	0.828**	1.0	0.925**
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-105.000	290.680	262.240	249.800
	Covariance	-4.375	12.112	10.927	10.408
	N	25	25	25	25
Number of Leaves *A. adianthifolia*	Pearson Correlation	-0.882**	0.904**	0.925**	1.0
	P (2-tailed)	<0.001	<0.001	<0.001	<0.001
	Sum of Squares and Cross-products	-104.000	326.600	249.800	278.000
	Covariance	-4.333	13.608	10.408	11.583
	N	25	25	25	25

**Correlation is significant at P<0.01 (2-tailed).
[#]Weeks After Planting (WAP).

Appendix 4.31: ANOVA of leguminous tree species shoot and root biomass (total plant biomass) at 16 WAP[#]

		Sum of Squares	df	Mean Square	F	P
Soil with *P. pterocarpum* number of shoots	Between Groups	4063.313	4	1015.828	298.247	<0.001
	Within Groups	34.060	10	3.406		
	Total	4097.373	14			
Soil with *P. pterocarpum* number of roots	Between Groups	152.703	4	38.176	26.684	<0.001
	Within Groups	14.307	10	1.431		
	Total	167.009	14			
Soil with *A. odoratissima* Number of shoots	Between Groups	3553.429	4	888.357	72.432	<0.001
	Within Groups	122.647	10	12.265		
	Total	3676.076	14			
Soil with *A. odoratissima* number of roots	Between Groups	90.320	4	22.580	20.065	<0.001
	Within Groups	11.253	10	1.125		
	Total	101.573	14			
Soil with *A. adianthifolia* number of shoots	Between Groups	2851.496	4	712.874	355.135	<0.001
	Within Groups	20.073	10	2.007		
	Total	2871.569	14			
Soil with *A. adianthifolia* number of roots	Between Groups	130.287	4	32.572	34.973	<0.001
	Within Groups	9.313	10	0.931		
	Total	139.600	14			

[#]Weeks After Planting (WAP).

Appendix 4.32: ANOVA of microbial counts in crude oil-contaminated soil planted with leguminous tree species (LTS) at 16 WAP[#]

		Sum of Squares	df	Mean Square	F	P
Soil with *P. pterocarpum* Number of Bacteria	Between Groups	3606.267	4	901.567	5.807	0.011
	Within Groups	1552.667	10	155.267		
	Total	5158.933	14			
Soil with *P. pterocarpum* Number of Fungi	Between Groups	1430.267	4	357.567	17.760	<0.001
	Within Groups	201.333	10	20.133		
	Total	1631.600	14			
Soil with *A. odoratissima* Number of Bacteria	Between Groups	1004.933	4	251.233	3.727	0.042
	Within Groups	674.000	10	67.400		
	Total	1678.933	14			
Soil with *A. odoratissima* Number of Fungi	Between Groups	508.667	4	127.167	5.313	0.015
	Within Groups	239.333	10	23.933		
	Total	748.000	14			
Soil with *A. adianthifolia* Number of Bacteria	Between Groups	868.933	4	217.233	2.042	0.164
	Within Groups	1064.000	10	106.400		
	Total	1932.933	14			
Soil with *A. adianthifolia* Number of Fungi	Between Groups	460.267	4	115.067	3.977	0.035
	Within Groups	289.333	10	28.933		
	Total	749.600	14			

[#]Weeks After Planting (WAP).

Appendix 4.33: ANOVA of TPH in crude oil-contaminated soil remediated with leguminous tree species (LTS) at 16 WAP[#]

		Sum of Squares	df	Mean Square	F	P
TPH Non Planted soil	Between Groups	1268.419	3	422.806	1.437	0.302
	Within Groups	2353.907	8	294.238		
	Total	3622.326	11			
TPH Soil with *P. pterocarpum*	Between Groups	270.564	3	90.188	11.228	0.003
	Within Groups	64.260	8	8.033		
	Total	334.824	11			
TPH Soil with *A. odoratissima*	Between Groups	579.413	3	193.138	9.813	0.005
	Within Groups	157.460	8	19.682		
	Total	736.872	11			
TPH Soil with *A. adianthifolia*	Between Groups	1806.051	3	602.017	12.201	0.002
	Within Groups	394.727	8	49.341		
	Total	2200.778	11			

[#]Weeks After Planting (WAP).

4.34: Correlations analysis between TPHs in crude oil-contaminated soil planted with leguminous tree species (LTS) at 16 WAP[#]

		Treatments	TPH Soil with *P. pterocarpum*	TPH Soil with *A. odoratissima*	TPH Soil with *A. adianthifolia*
Treatments	Pearson Correlation	1.0	0.818**	0.731**	0.746**
	P (2-tailed)	<0.001	<0.001	0.007	0.005
	N	12	12	12	12
TPH Soil with *P. pterocarpum*	Pearson Correlation	0.818**	1.0	0.640*	0.870**
	P (2-tailed)	<0.001	<0.001	0.025	<0.001
	N	12	12	12	12
TPH Soil with *A. odoratissima*	Pearson Correlation	0.731**	0.640*	1.0	0.511
	P (2-tailed)	0.007	0.025	<0.005	0.090
	N	12	12	12	12
TPH Soil with *A. adianthifolia*	Pearson Correlation	0.746**	0.870**	0.511	1.0
	P (2-tailed)	0.005	<0.001	0.090	<0.001
	N	12	12	12	12

**Correlation is significant at P <0.01 (2-tailed).

*Correlation is significant at P <0.05 (2-tailed).

[#]Weeks After Planting (WAP).

Appendix 5.1: ANOVA between oil-contamination and 10 g tropical kaolinite sorption rate

Dependent Variable: Sorption

	Sum of Squares	df	Mean Square	F	P
Between Groups	8343.221	4	2085.805	2046.866	<0.001
Within Groups	20.380	20	1.019		
Total	8363.602	24			

Appendix 5.2: Multiple comparisons of oil-contamination and mean sorption rate

Dependent Variable: Sorption

	(I) Treatments	(J) Treatments	Mean Difference (I-J)	SE	P	95% Confidence Interval	
						Lower Boundary	Upper Boundary
LSD	0 ml Control	25 ml Low contamination	51.4180*	0.63844	<0.001	50.0862	52.7498
		50 ml Moderate contamination	46.8580*	0.63844	<0.001	45.5262	48.1898
		75 ml High contamination	37.3380*	0.63844	<0.001	36.0062	38.6698
		100 ml Very high contamination	27.4980*	0.63844	<0.001	26.1662	28.8298
	25 ml Low contamination	0 ml Control	-51.4180*	0.63844	<0.001	-52.7498	-50.0862
		50 ml Moderate contamination	-4.5600*	0.63844	<0.001	-5.8918	-3.2282
		75 ml High contamination	-14.0800*	0.63844	<0.001	-15.4118	-12.7482
		100 ml Very high contamination	-23.9200*	0.63844	<0.001	-25.2518	-22.5882
	50 ml Moderate contamination	0 ml Control	-46.8580*	0.63844	<0.001	-48.1898	-45.5262
		25 ml Low contamination	4.5600*	0.63844	<0.001	3.2282	5.8918
		75 ml High contamination	-9.5200*	0.63844	<0.001	-10.8518	-8.1882
		100 ml Very high contamination	-19.3600*	0.63844	<0.001	-20.6918	-18.0282
	75 ml High contamination	0 ml Control	-37.3380*	0.63844	<0.001	-38.6698	-36.0062
		25 ml Low contamination	14.0800*	0.63844	<0.001	12.7482	15.4118
		50 ml Moderate contamination	9.5200*	0.63844	<0.001	8.1882	10.8518
		100 ml Very high contamination	-9.8400*	0.63844	<0.001	-11.1718	-8.5082
	100 ml Very high contamination	0 ml Control	-27.4980*	0.63844	<0.001	-28.8298	-26.1662
		25 ml Low contamination	23.9200*	0.63844	<0.001	22.5882	25.2518
		50 ml Moderate contamination	19.3600*	0.63844	<0.001	18.0282	20.6918
		75 ml High contamination	9.8400*	0.63844	<0.001	8.5082	11.1718

Based on observed means.

The error term is Mean Square (Error) = 1.019.

*The mean difference is significant at P <0.05.

Appendix 5.3: Correlations coefficients between oil-contamination and 10 g tropical kaolinite sorption rate

		Sorption	Treatments
Pearson Correlation	Sorption	1.000	-0.316
	Treatments	-0.316	1.000
P (1-tailed)	Sorption	0.005	0.062
	Treatments	0.062	0.005
N	Sorption	25	25
	Treatments	25	25

Summary of correlation between oil-contamination and 10 g tropical kaolinite sorption rate

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson statistic
					R Square Change	F Change	df1	df2	Sig. F Change	
1.0	0.316[a]	0.100	0.061	18.900	0.100	2.558	1.0	23	0.123	0.372

a. Predictors: (Constant), Treatments.

b. Dependent Variable: Adsorption.

Appendix 5.4: ANOVA between oil-contamination and 20 g tropical kaolinite sorption rate

Dependent Variable: Sorption

	Sum of Squares	df	Mean Square	F	P
Between Groups	10526.164	4	2631.541	3642.745	<0.001
Within Groups	14.448	20	0.722		
Total	10540.612	24			

Appendix 5.5: Multiple comparisons of oil-contamination and mean sorption rate

Dependent Variable: Adsorption

	(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	P	95% Confidence Interval	
						Lower Boundary	Upper Boundary
LSD	O ml Control	25 ml Low contamination	56.06600*	0.53755	<0.001	54.9447	57.1873
		50 ml Moderate contamination	54.24600*	0.53755	<0.001	53.1247	55.3673
		75 ml High contamination	46.46600*	0.53755	<0.001	45.3447	47.5873
		100 ml Very high contamination	36.70600*	0.53755	<0.001	35.5847	37.8273
	25 ml Low contamination	0 ml Control	-56.06600*	0.53755	<0.001	-57.1873	-54.9447
		50 ml Moderate contamination	-1.82000*	0.53755	0.003	-2.9413	-.6987
		75 ml High contamination	-9.60000*	0.53755	<0.001	-10.7213	-8.4787
		100 ml Very high contamination	-19.36000*	0.53755	<0.001	-20.4813	-18.2387
	50 ml Moderate contamination	0 ml Control	-54.24600*	0.53755	<0.001	-55.3673	-53.1247
		25 ml Low contamination	1.82000*	0.53755	0.003	.6987	2.9413
		75 ml High contamination	-7.78000*	0.53755	<0.001	-8.9013	-6.6587
		100 ml Very high contamination	-17.54000*	0.53755	<0.001	-18.6613	-16.4187
	75 ml High contamination	0 ml Control	-46.46600*	0.53755	<0.001	-47.5873	-45.3447
		25 ml Low contamination	9.60000*	0.53755	<0.001	8.4787	10.7213
		50 ml Moderate contamination	7.78000*	0.53755	<0.001	6.6587	8.9013
		100 ml Very high contamination	-9.76000*	0.53755	<0.001	-10.8813	-8.6387
	100 ml Very high contamination	0 ml Control	-36.70600*	0.53755	<0.001	-37.8273	-35.5847
		25 ml Low contamination	19.36000*	0.53755	<0.001	18.2387	20.4813
		50 ml Moderate contamination	17.54000*	0.53755	<0.001	16.4187	18.6613
		75 ml High contamination	9.76000*	0.53755	<0.001	8.6387	10.8813

*. The mean difference is significant P <0.05.

Appendix 5.6: Correlation cofficients between oil-contamination and 20 g tropical kaolinite sorption rate

		Sorption	Treatments
Pearson Correlation	Sorption	1.000	-0.439
	Treatments	-0.439	1.000
Sig. (1-tailed)	Sorption	0.005	0.014
	Treatments	0.014	0.005
N	Sorption	25	25
	Treatments	25	25

Summary of correlation between oil-contamination and 20 g tropical kaolinite sorption rate

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson statistic
					R Square Change	F Change	df1	df2	Sig. F Change	
1	0.439[a]	0.193	0.158	19.229	0.193	5.506	1.0	23	0.028	0.342

a. Predictors: (Constant), Treatments

b. Dependent Variable: Adsorption.

Appendix 5.7: Correlation coefficients between oil-contamination and sorption rates of 10 g and 20 g tropical kaolinite

		10 g kaolinite	20 g kaolinite
	Pearson Correlation	1.0	0.975*
	P (2-tailed)		0.025
10 g kaolinite	Sum of Squares and Cross-products	350.231	252.503
	Covariance	116.744	84.168
	N	4	4
	Pearson Correlation	0.975*	1.0
	P (2-tailed)	0.025	
20 g kaolinite	Sum of Squares and Cross-products	252.503	191.432
	Covariance	84.168	63.811
	N	4	4

*Correlation is significant P <0.05.